

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF THE RECORDING
OF A CHANGE(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

Monk, Jonathan Paul
Baldwin Shelston Waters
NCR Building
342 Lambton Quay
Wellington_6001
NOUVELLE-ZÉLANDE

Date of mailing (day/month/year)

15 January 2001 (15.01.01)

Applicant's or agent's file reference

25426 MRB

IMPORTANT NOTIFICATION

International application No.

PCT/NZ99/00228

International filing date (day/month/year)

24 December 1999 (24.12.99)

1. The following indications appeared on record concerning:

☐

the applicant

☐

the inventor

☒

the agent

☐

the common representative

Name and Address

BENNETT, Michael, Roy
West-Walker Bennett
Mobil on the Park
157 Lambton Quay
Wellington
New Zealand

State of Nationality

State of Residence

Telephone No.

64 4 499 9058

Facsimile No.

64 4 499 9306

Teleprinter No.

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☐

the person

☐

the name

☐

the address

☐

the nationality

☐

the residence

Name and Address

Monk, Jonathan Paul
Baldwin Shelston Waters
NCR Building
342 Lambton Quay
Wellington_6001
New Zealand

State of Nationality

State of Residence

Telephone No.

64 4 499 9058

Facsimile No.

64 4 499 9306

Teleprinter No.

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

☒

the receiving Office

☐

the designated Offices concerned

☐

the International Searching Authority

☒

the elected Offices concerned

☒

the International Preliminary Examining Authority

☐

other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Dominique DELMAS

Telephone No.: (41-22) 338.83.38

F ENT COOPERATION TREA

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Assistant Commissioner for Patents
 United States Patent and Trademark
 Office
 Box PCT
 Washington, D.C.20231
 ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 22 August 2000 (22.08.00)	
International application No. PCT/NZ99/00228	Applicant's or agent's file reference 25426 MRB
International filing date (day/month/year) 24 December 1999 (24.12.99)	Priority date (day/month/year) 24 December 1998 (24.12.98)
Applicant FRASER, John, David et al	

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International Preliminary Examining Authority on:
 21 July 2000 (21.07.00)

☐ in a notice effecting later election filed with the International Bureau on:

2. The election ☒ was
☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer Manu Berrod Telephone No.: (41-22) 338.83.38
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REC'D 27 FEB 2001

PCT

14

Applicant's or agent's file reference JM/503288-142	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).	
International Application No. PCT/NZ99/00228	International Filing Date (day/month/year) 24 December 1999	Priority Date (day/month/year) 24 December 1998
International Patent Classification (IPC) or national classification and IPC Int. Cl. ⁷ C07K 14/315, 16/12; C07H 19/00; C12N 1/20; C12Q 1/68; A61K 35/74, 39/09		
Applicant AUCKLAND UNISERVICES LIMITED et al		

1.	This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.																								
2.	This REPORT consists of a total of 6 sheets, including this cover sheet. <input type="checkbox"/> This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT). These annexes consist of a total of sheet(s).																								
3.	This report contains indications relating to the following items: <table border="0"> <tr> <td>I</td> <td><input checked="" type="checkbox"/></td> <td>Basis of the report</td> </tr> <tr> <td>II</td> <td><input type="checkbox"/></td> <td>Priority</td> </tr> <tr> <td>III</td> <td><input type="checkbox"/></td> <td>Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</td> </tr> <tr> <td>IV</td> <td><input type="checkbox"/></td> <td>Lack of unity of invention</td> </tr> <tr> <td>V</td> <td><input checked="" type="checkbox"/></td> <td>Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</td> </tr> <tr> <td>VI</td> <td><input checked="" type="checkbox"/></td> <td>Certain documents cited</td> </tr> <tr> <td>VII</td> <td><input type="checkbox"/></td> <td>Certain defects in the international application</td> </tr> <tr> <td>VIII</td> <td><input type="checkbox"/></td> <td>Certain observations on the international application</td> </tr> </table>	I	<input checked="" type="checkbox"/>	Basis of the report	II	<input type="checkbox"/>	Priority	III	<input type="checkbox"/>	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability	IV	<input type="checkbox"/>	Lack of unity of invention	V	<input checked="" type="checkbox"/>	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement	VI	<input checked="" type="checkbox"/>	Certain documents cited	VII	<input type="checkbox"/>	Certain defects in the international application	VIII	<input type="checkbox"/>	Certain observations on the international application
I	<input checked="" type="checkbox"/>	Basis of the report																							
II	<input type="checkbox"/>	Priority																							
III	<input type="checkbox"/>	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability																							
IV	<input type="checkbox"/>	Lack of unity of invention																							
V	<input checked="" type="checkbox"/>	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement																							
VI	<input checked="" type="checkbox"/>	Certain documents cited																							
VII	<input type="checkbox"/>	Certain defects in the international application																							
VIII	<input type="checkbox"/>	Certain observations on the international application																							

Date of submission of the demand 21 July 2000	Date of completion of the report 14 February 2001
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer  IAN DOWD Telephone No. (02) 6283 2273

I. Basis of the report**1. With regard to the elements of the international application:***

- ☒ the international application as originally filed.
- ☐ the description, pages , as originally filed,
 pages , filed with the demand,
 pages , received on with the letter of
- ☐ the claims, pages , as originally filed,
 pages , as amended (together with any statement) under Article 19,
 pages , filed with the demand,
 pages , received on with the letter of
- ☐ the drawings, pages , as originally filed,
 pages , filed with the demand,
 pages , received on with the letter of
- ☐ the sequence listing part of the description:
 pages , as originally filed
 pages , filed with the demand
 pages , received on with the letter of

2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language which is:

- ☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, was on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☒ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages
- ☐ the claims, Nos.
- ☐ the drawings, sheets/fig.

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).

** Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**1. Statement**

Novelty (N)	Claims 1-30	YES
	Claims	NO
Inventive step (IS)	Claims 1-30	YES
	Claims	NO
Industrial applicability (IA)	Claims 1-30	YES
	Claims	NO

2. Citations and explanations (Rule 70.7)

The following documents identified in the International Search Report have been considered for the purposes of this report:

- D1 Journal of Experimental medicine (1999) 189(1), 89-101
"Identification and characterization of novel superantigens from *Streptococcus pyogenes*" Proft, T et al
- D2 Database GenPept, Accession No. CAB51744, Authors: Gerlach, D, Wagner, M, Fleischer, B, Schmidt, KH, Vettermann, S, Reichardt, W.
Submitted 29 July 1999.
- D3 Database GenPept, Accession No. CAB51332, Authors: Gerlach, D, Wagner, M, Fleischer, B, Schmidt, KH, Vettermann, S, Reichardt, W.
Submitted 19 July 1999.
- D4 Database GenPept, Accession No. CAB51142, Authors: Gerlach, D, Wagner, M, Fleischer, B, Schmidt, KH, Vettermann, S, Reichardt, W.
Submitted 19 July 1999.
- D5 Infection and Immunity (1998) 66(7), 3337-3348
"Identification and Characterization of Staphylococcal Enterotoxin Types G and I from *Staphylococcus aureus* "
Munson, SH et al
- D6 Molecular Microbiology (1998) 29(2), 527-543
"The gene for toxic shock toxin is carried by a family of mobile pathogenicity islands in *staphylococcus aureus*". Lindsay, JA et al.
- D7 Infection and Immunity (1998) 56(9), 2518-2520
"Nucleotide Sequence of Streptococcal Pyrogenic Exotoxin Type C"
Goshorn, SC and Schlievert, PM

Continued on Supplemental Sheet

VI. Certain documents cited**1. Certain published documents (Rule 70.10)**

Application No. Patent No.	Publication date (day/month/year)	Filing date (day/month/year)	Priority date (valid claim) (day/month/year)
P,X WO 99/27889	10 June 1999	1 December 1998	2 December 1997

2. Non-written disclosures (Rule 70.9)

Kind of non-written disclosure	Date of non-written disclosure (day/month/year)	Date of written disclosure referring to non- written disclosure (day/month/year)
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Supplemental Box

(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of Box V

D8 Database Swiss-Prot, Accession No. SPEC_STRPY
& Infection and Immunity (1998) 56(9), 2518-2520
& Infection and Immunity (1992) 60: 3513-3517
& Nat Struct Biol (1997) 4: 635-643

D9 Database GenPept, Accession No. AAB 59091,
& Infection and Immunity (1998) 56(9), 2518-2520
& Infection and Immunity (1992) 60: 3513-3517

D10 WO 99/27889 (10 June 1999) IDAHO RESEARCH FOUNDATION INC

Documents D1, D2, D3, and D4 have an earlier publication date than the priority date claimed in the international application. The International Preliminary Searching Authority was unable to view the priority document of the international application to ascertain priority entitlement. However, this report is established on the assumption that the application enjoys the earlier date.

Documents D1, D2, D3, and D4 are excluded for the purposes of considering Novelty or Inventive Step due to the assumption explained above. However, if a priority issue with the application, eg the application is not entitled to the earlier priority, arises then these documents would become relevant and should be considered for the purposes of novelty and inventive step.

With regard to the document D10 listed in Box VI under "certain documents cited", these are documents published prior to the international filing date but later than the priority date claimed but which would otherwise be considered to be of particular relevance.

Under the PCT, novelty is considered only in respect of documents published before the priority date. The relevance of a document published after the priority date is dependent upon national law. Such documents are excluded from consideration in preliminary examination, under the PCT Guidelines but have been included here for information.

NOVELTY and INVENTIVE STEP

Documents D5 and D6 disclose polypeptide sequence listings that have 35% and 34%, respectively, identity to sequence ID No. 2 of the application. Documents D7, D8 and D9 disclose polypeptide sequence listings that have 55.5%, 55.5% and 56%, respectively, identity to sequence ID No. 8 of the application. The description of the invention in the application does not give an indication of the percentage identity the claimed polypeptide sequences need as a minimum to retain functional equivalence. Therefore bearing this in mind, the documents cited herein are potentially novelty destroying if the above identified identity affords functional equivalence.

However, the applicant has argued that the citations identified disclose sequences of other known bacterial superantigens having different functional properties. The sequences, they argue, encode for proteins with significantly different functional properties, host specificities and potential disease associations. Given these arguments, this Preliminary Examining Authority acknowledges novelty and inventive step for the claims relating to sequence ID No.s 2 and 8. Consequently, sequence ID No.s 1 and 7 relating to the nucleotide of Sequence ID No.s 2 and 8, respectively, are also novel and inventive.

Continued on Supplemental Sheet

Supplemental Box

(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of Box V

Documents D5, D6, D7, D8, and D9 do not disclose Sequence ID No.s 4 or 6 relating to polypeptide. Therefore claims relating to these sequence ID's are novel. Furthermore, documents D5, D6, D6, D8, and D9 do not lead a person skilled in the art to arrive at Sequence ID No.s 4 or 6. Therefore claims relating to these sequence ID No.s 4 or 6 are non-obvious and possess an inventive step. Consequently, sequence ID No.s 3 and 5 relating to the nucleotide of Sequence ID No.s 4 and 6, respectively, are also novel and inventive.

INDUSTRIAL APPLICABILITY

Rule 67 lists the subject matter which under Article 34(4)(a)(i) an international preliminary examination is not required to be carried out. At item (iv) it specifies methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods, as such matter. However the agreement between WIPO and Australia further qualifies this by excepting from exclusion any subject matter which is examined under national grant procedures. Claim 30 has nonetheless been considered because the identified subject matter does not contravene Australian law. Consequently, claims 1-30 relate to matter which is considered to meet the requirement of Article 33(4).

INTERNATIONAL COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 25426 MRB	<div style="display: flex; justify-content: space-between;"> <div style="width: 30%; text-align: center;">FOR FURTHER ACTION</div> <div style="width: 70%;">see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.</div> </div>	
International application No. PCT/NZ99/00228	International filing date (<i>day/month/year</i>) 24 December 1999	(Earliest) Priority Date (<i>day/month/year</i>) 24 December 1998
Applicant AUCKLAND UNISERVICES LIMITED		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 4 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

- a. With regard to the language, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

- b. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of the sequence listing:

☒ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☐ Certain claims were found unsearchable (See Box I).

3. ☐ Unity of invention is lacking (See Box II).

4. With regard to the title, ☒ the text is approved as submitted by the applicant.
☐ the text has been established by this Authority to read as follows:

5. With regard to the abstract, ☒ the text is approved as submitted by the applicant
☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the drawings to be published with the abstract is Figure No.

☐ as suggested by the applicant.

☐ because the applicant failed to suggest a figure

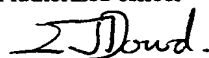
☐ because this figure better characterizes the invention

☒ None of the figures

INTERNATIONAL SEARCH REPORT

International application No.

PCT/NZ99/00228

A. CLASSIFICATION OF SUBJECT MATTER																						
Int. Cl. ⁷ : C07K 14/315, C07K 16/12, C07H 19/00, C12N 1/20, C12Q 1/68, A61K 35/74, A61K 39/09																						
According to International Patent Classification (IPC) or to both national classification and IPC																						
B. FIELDS SEARCHED																						
Minimum documentation searched (classification system followed by classification symbols)																						
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched																						
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) STN: File WPIDS Keywords used: "superantigen or super(w) antigen" and "streptococ?" ANGIS Database: Sequence ID No's 2, 4, 6, and 8.																						
C. DOCUMENTS CONSIDERED TO BE RELEVANT																						
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.																				
P,X	Journal of Experimental medicine (1999) 189(1), 89-101 "Identification and characterization of novel superantigens from <i>Streptococcus pyogenes</i> " Proft, T et al	1-30																				
P,X	Database GenPept, Accession No. CAB51744, Authors: Gerlach, D, Wagner, M, Fleischer, B, Schmidt, KH, Vettermann, S, Reichardt, W. Submitted 29 July 1999.	1, 2, 6, 7, 14-20, 24, 25, 29, 30																				
P,X	Database GenPept, Accession No. CAB51332, Authors: Gerlach, D, Wagner, M, Fleischer, B, Schmidt, KH, Vettermann, S, Reichardt, W. Submitted 19 July 1999.	1, 2, 6, 7, 14-20, 24, 25, 29, 30																				
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C <input checked="" type="checkbox"/> See patent family annex																						
<p>* Special categories of cited documents:</p> <table border="0"> <tr> <td>"A"</td> <td>document defining the general state of the art which is not considered to be of particular relevance</td> <td>"T"</td> <td>later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</td> </tr> <tr> <td>"E"</td> <td>earlier application or patent but published on or after the international filing date</td> <td>"X"</td> <td>document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</td> </tr> <tr> <td>"L"</td> <td>document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</td> <td>"Y"</td> <td>document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</td> </tr> <tr> <td>"O"</td> <td>document referring to an oral disclosure, use, exhibition or other means</td> <td>"&"</td> <td>document member of the same patent family</td> </tr> <tr> <td>"P"</td> <td>document published prior to the international filing date but later than the priority date claimed</td> <td></td> <td></td> </tr> </table>			"A"	document defining the general state of the art which is not considered to be of particular relevance	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	"E"	earlier application or patent but published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	"O"	document referring to an oral disclosure, use, exhibition or other means	"&"	document member of the same patent family	"P"	document published prior to the international filing date but later than the priority date claimed		
"A"	document defining the general state of the art which is not considered to be of particular relevance	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention																			
"E"	earlier application or patent but published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone																			
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art																			
"O"	document referring to an oral disclosure, use, exhibition or other means	"&"	document member of the same patent family																			
"P"	document published prior to the international filing date but later than the priority date claimed																					
Date of the actual completion of the international search XX March 2000		Date of mailing of the international search report - 8 MAY 2000																				
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustalia.gov.au Facsimile No. (02) 6285 3929		Authorized officer  IAN DOWD Telephone No : (02) 6283 2273																				

INTERNATIONAL SEARCH REPORT

International application No.

PCT/NZ99/00228

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	Database GenPept, Accession No. CAB51142, Authors: Gerlach, D, Wagner, M, Fleischer, B, Schmidt, KH, Vettermann, S, Reichardt, W. Submitted 19 July 1999.	1, 2, 6, 7, 14-20, 24, 25, 29, 30
X	Infection and Immunity (1998) 66(7), 3337-3348 " Identification and Characterization of Staphylococcal Enterotoxin Types G and I from <i>Staphylococcus aureus</i> " Munson, SH et al.	1, 2, 6, 7, 14-20, 24, 25, 29, 30
X	Molecular Microbiology (1998) 29(2), 527-543 "The gene for toxic shock toxin is carried by a family of mobile pathogenicity islands in <i>staphylococcus aureus</i> ". Lindsay, JA et al.	1, 2, 6, 7, 14-20, 24, 25, 29, 30
X	Infection and Immunity (1998) 56(9), 2518-2520 " Nucleotide Sequence of Streptococcal Pyrogenic Exotoxin Type C" Goshorn, SC and Schlievert, PM.	1, 5, 12-20, 23, 24, 28-30
X	Database Swiss-Prot, Accession No. SPEC_STRPY & Infection and Immunity (1998) 56(9), 2518-2520 & Infection and Immunity (1992) 60: 3513-3517 & Nat Struct Biol (1997) 4: 635-643	1, 5, 12-20, 23, 24, 28-30
X	Database GenPept, Accession No. AAB 59091, & Infection and Immunity (1998) 56(9), 2518-2520 & Infection and Immunity (1992) 60: 3513-3517	1, 5, 12-20, 23, 24, 28-30
P,X	WO 99/27889 (10 June 1999) IDAHO RESEARCH FOUNDATION INC See claim 3 in particular.	1, 3, 4, 8-11, 14-19, 21-22, 24, 26-27, 29-30

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/NZ99/00228

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report	Patent Family Member
WO 99/27889	
END OF ANNEX	

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

SUPERANTIGENS**TECHNICAL FIELD**

- 5 This invention relates to superantigens, and to their use, including in diagnosis and/or treatment of disease.

BACKGROUND ART

- 10 Bacterial superantigens are the most potent T cell mitogens known. They stimulate large numbers of T cells by directly binding to the side of the MHC class II and T cell Receptor (TcR) molecules. Because they override the normally exquisite MHC restriction phenomenon of T cell antigen recognition, they are prime candidates for either causing the onset of autoimmune diseases or exacerbating an existing
15 autoimmune disorder.

The applicants have identified genes coding for four novel superantigens from *S. pyogenes*. It is broadly to these superantigens and polynucleotides encoding them that the present invention is directed.

20

SUMMARY OF THE INVENTION

In one aspect the invention provides a superantigen selected from any one of SMEZ-2, SPE-G, SPE-H and SPE-J, or a functionally equivalent variant thereof.

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In a further aspect the invention provides a polynucleotide molecule comprising a sequence encoding a superantigen chosen from SMEZ-2, SPE-G, SPE-H, SPE-J, or a functionally equivalent variant thereof.

- 30 In another aspect of the invention there is provided a method of subtyping *Streptococci* on the basis of superantigen genotype comprising detection of the presence of any or all of the above four superantigens or the corresponding polynucleotides.

In a further aspect the invention provides a construct comprising any of the above superantigens (or superantigen variants) bound to a cell-targeting molecule, which is preferably a tumour-specific antibody.

- 5 In yet a further aspect, the invention provides a pharmaceutical composition for therapy or prophylaxis comprising a superantigen or superantigen variant as described above linked to cell targeting molecule.

Other aspects of the invention will be apparent from the description provided below,
10 and from the appended claims.

DESCRIPTION OF DRAWINGS

While the invention is broadly defined above, it further includes embodiments of
15 which the following description provides examples. It will also be better understood with reference to the following drawings:

Fig 1: Multiple alignment of superantigen protein sequences.

- 20 The protein sequence of mature toxins were aligned using the PileUp programme on the GCG package. Regions of high sequence identity are in black boxes. The boxes below the sequences indicate the structural elements of SPE-C, as determined from the crystal structure (Roussel et al 1997 Nat. Struct. Biol. 4 no8:635-43). Regions with highest homology correspond to the $\beta 4$, $\beta 5$, $\alpha 4$ and $\alpha 5$ regions in SPE-C. The
25 clear box near the C-terminus represents a primary zinc binding motif, a common feature of all toxins shown. The arrows on top of the sequence alignment show the regions of sequence diversity between SMEZ and SMEZ-2.

Figure 2: The nucleotide sequence of the portion of the smeZ-2 gene (SEQ ID NO. 1)
30 coding the mature SMEZ-2 superantigen (SEQ ID NO. 2).

Figure 3: The nucleotide sequence of the portion of the spe-g gene (SEQ ID NO. 3) coding the mature SPE-G superantigen (SEQ ID NO. 4).

Figure 4: The nucleotide sequence of the portion of the spe-h gene (SEQ ID NO. 5) coding the mature SPE-H superantigen (SEQ ID NO. 6).

Figure 5: The nucleotide sequence of the portion of the spe-j gene (SEQ ID NO. 7) coding part of the mature SPE-J superantigen (SEQ ID NO. 8).

Figure 6: Gel electrophoresis of the purified recombinant toxins.

A. Two micrograms of purified recombinant toxin were run on a 12.5% SDS-polyacrylamide gel to show the purity of the preparations; B. Two micrograms of purified recombinant toxin were run on an isoelectric focusing gel (5.5% PAA, pH 5-8). The isoelectric point (IEP) of rSMEZ-2, rSPE-G and rSPE-H is similar and was estimated at pH 7-8. The IEP of rSMEZ was estimated at pH 6-6.5.

Figure 7: Stimulation of human T cells with recombinant toxins.

PBLs were isolated from human blood samples and incubated with varying concentrations of recombinant toxin. After 3d, 0.1 μ Ci [3 H]-thymidine was added and cells were incubated for another 24h, before harvested and counted on a gamma counter. O, unstimulated; \blacktriangle , rSMEZ; \checkmark , rSMEZ-2; \blacklozenge , rSPE-G; \blacksquare , rSPE-H.

Figure 8: Jurkat cell assay

Jurkat cells (bearing a V β 8 TcR) and LG-2 cells were mixed with varying concentrations of recombinant toxin and incubated for 24h, before SeI cells were added. After 1d, 0.1 μ Ci [3 H]-thymidine was added and cells were counted after another 24h. The V β 8 targeting SEE was used as a positive control. The negative control was SEA. Both SMEZ and SMEZ-2 were potent stimulators of Jurkat cells, indicating their ability to specifically target V β 8 bearing T cells. O, unstimulated; \blacktriangle , rSEA; \checkmark , rSEE; \blacklozenge , rSMEZ; \blacksquare , rSMEZ-2.

Figure 9: Zinc dependent binding of SMEZ-2 to LG-2 cells

LG-2 cells were incubated in duplicates with 1 ng of ^{125}I labelled rSMEZ-2 and increasing amounts of unlabeled toxin at 37°C for 1h, and then the cells were washed and counted.

- 5 O, incubation in media; ▲, incubation in media plus 1mM EDTA; Ž, incubation in media plus 1 mM EDTA, 2 mM ZnCl_2 .

Figure 10: Scatchard analysis of SMEZ-2 binding to LG-2 cells

- 10 One nanogram ^{125}I -labeled rSMEZ-2 was incubated in duplicates with LG-2 cells and a 2-fold dilution series of cold toxin (10 μg to 10 pg). After 1h, cells were washed and counted. Scatchard plots were performed as described by Cunningham et al 1989 Science 243:1330-1336.

- 15 Figure 11: Summary of competitive binding experiments.

Efficiency of each labelled toxin to compete with a 10,000-fold molar excess of any other unlabeled toxin for binding to LG-2 cells. □, no competition; ▤, 25% competition; ▥, 50% competition; ▦, 75% competition; ■, 100% competition.

- 20 The results within the boxes are at the bottom right have previously been published (Li et al. 1997).

Figure 12: Competition binding study with SMEZ-2.

- 25 LG-2 cells were incubated in duplicates with 1 ng of ^{125}I -labeled rSMEZ-2 and increasing amounts of unlabeled rSMEZ-2, rSEA, rSEB, rTSST or rSPE-C. After 1h cells were washed and counted.

O, rSMEZ-2; ▲, rSEA; Ž, rSEB; ▤ rTSST; ◆, rSPE-C.

- 30 Figure 13: Southern blot analysis of genomic DNA with radiolabeled smeZ. HINDIII digested genomic DNA from various *Streptococcus* isolates was hybridized with a radiolabeled smeZ probe. Band A is a 1953 bp HindIII DNA fragment that carries the smeZ gene. Bands B and C are DNA fragments of about 4 kbp and 4.2 kbp, respectively, which both carry a smeZ like region. 1, *S. pyogenes* reference strain
35 (ATCC 700294, M1 type); 2, isolate 9639 (MNT); 3, isolate 11789 (MNT); 4, isolate

11152 (PT2612 type); 5, isolate RC4063 (group C streptococcus); 6, isolate 11070 (emm65 type); 7, DNA marker lane; 8, isolate 4202 (NZ5118/M92 type); 9, isolate 94/229 (M49 type); 10, isolate 11610 (emm57 type); 11, isolate 95/127 (NZ1437/M89 type); 12, isolate 94/330 (M4 type).

5

DESCRIPTION OF THE INVENTION

The focus of the invention is the identification of four superantigens (SPE-G, SPE-H, SPE-J and SMEZ-2) and the corresponding polynucleotides which encode them.

10

Figure 1 shows the amino acid sequences of the above four superantigens together with those of previously identified superantigens SMEZ, SPE-C and SEA.

Of the four superantigens SPE-G, SPE-H, SPE-J and SMEZ-2, the latter is perhaps of greatest interest.

15

The smeZ-2 gene which encodes SMEZ-2 was identified in an experiment designed to produce recombinant SMEZ protein from *S. pyogenes* 2035 genomic DNA. A full length smeZ gene was isolated from the strain but the DNA sequence of the smeZ gene of strain 2035 showed nucleotide changes in 36 positions (or 5%) compared to smeZ from strain M1 (Fig. 1). The deduced protein sequences differed in 17 amino acid residues (or 8.1%). This difference establishes this as a new gene, smeZ-2, and the encoded protein as a new superantigen, SMEZ-2.

20

The most significant difference between SMEZ and SMEZ-2 is an exchanged pentapeptide sequence at position 96-100, where the EEPMS sequence of SMEZ is converted to KTSIL in SMEZ-2 (Fig. 1). A second difference is at position 111-112, where an RR dipeptide is exchanged for GK in SMEZ-2. The remaining 10 different residues are spread over almost the entire primary sequence.

25

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Figure 2 shows the nucleotide sequence encoding mature SMEZ-2 and the deduced amino acid sequence.

Likewise, Figures 3 to 5 show the nucleotide sequence encoding mature SPE-G, SPE-H and SPE-J superantigens, respectively, together with their respective deduced amino acid sequences.

- 5 The invention is of course not restricted to superantigens/polynucleotides having the specific sequences of Figures 1 to 5. Instead, functionally equivalent variants are contemplated.

The phrase "functionally equivalent variants" recognises that it is possible to vary
10 the amino acid/nucleotide sequence of a peptide while retaining substantially equivalent functionality. For example, a peptide can be considered a functional equivalent of another peptide for a specific function if the equivalent peptide is immunologically cross-reactive with and has at least substantially the same function as the original peptide. The equivalent can be, for example, a fragment of
15 the peptide, a fusion of the peptide with another peptide or carrier, or a fusion of a fragment which additional amino acids. For example, it is possible to substitute amino acids in a sequence with equivalent amino acids using conventional techniques. Groups of amino acids normally held to be equivalent are:

- 20 (a) Ala, Ser, Thr, Pro, Gly;
(b) Asn, Asp, Glu, Gln;
(c) His, Arg, Lys;
(d) Met, Leu, Ile, Val; and
(e) Phe, Tyr, Trp.

25

Equally, nucleotide sequences encoding a particular product can vary significantly simply due to the degeneracy of the nucleic acid code.

Variants can have a greater or lesser degree of homology as between the variant
30 amino acid/nucleotide sequence and the original.

Polynucleotide or polypeptide sequences may be aligned, and percentage of identical nucleotides in a specified region may be determined against another sequence, using computer algorithms that are publicly available. Two exemplary algorithms
35 for aligning and identifying the similarity of polynucleotide sequences are the

- BLASTN and FASTA algorithms. The similarity of polypeptide sequences may be examined using the BLASTP algorithm. Both the BLASTN and BLASTP software are available on the NCBI anonymous FTP server (<ftp://ncbi.nlm.nih.gov>) under /blast/executables/. The BLASTN algorithm version 2.0.4 [Feb-24-1998], set to the
- 5 default parameters described in the documentation of variants according to the present invention. The use of the BLAST family of algorithms, including BLASTN and BLASTP, is described at NCBI's website at URL <http://www.ncbi.nlm.nih.gov/BLAST/newblast.html> and in the publication of Altschul, Stephen F., *et al.* (1997), "Gapped BLAST and PSI-BLAST: a new
- 10 generation of protein database search programs", *Nucleic Acids Res.* 25:3389-34023. The computer algorithm FASTA is available on the Internet at the ftp site <ftp://ftp.virginia.edu/pub/fasta/>. Version 2.0u4, February 1996, set to the default parameters described in the documentation and distributed with the algorithm, is also preferred for use in the determination of variants according to the present
- 15 invention. The use of the FASTA algorithm is described in W. R. Pearson and D. J. Lipman, "Improved Tools for Biological Sequence Analysis", *Proc. Natl. Acad. Sci. USA* 85:2444-2448 (1988) and W. R. Pearson, "Rapid and Sensitive Sequence Comparison with FASTP and FASTA", *Methods in Enzymology* 183:63-98 (1990).
- 20 The following running parameters are preferred for determination of alignments and similarities using BLASTN that contribute to E values (as discussed below) and percentage identity: Unix running command: `blastall -p blastn -d embldb -e 10 -G 1 -E 1 -r 2 -v 50 -b 50 -I queryseq -o results`; and parameter default values:
- p Program Name [String]
 - 25 -d Database [String]
 - e Expectation value (E) [Real]
 - G Cost to open a gap (zero invokes default behaviour) [Integer]
 - E Cost to extend a gap (zero invokes default behaviour) [Integer]
 - r Reward for a nucleotide match (blastn only) [Integer]
 - 30 -v Number of one-line descriptions (V) [Integer]
 - b Number of alignments to show (B) [Integer]
 - i Query File [File In]
 - o BLAST report Output File [File Out] Optional
- For BLASTP the following running parameters are preferred: `blastall -p blastp -d swissprot -e 10 -G 1 -E 1 -v 50 -b 50 -I queryseq -o results`
- 35

- p Program Name [String]
- d Database [String]
- e Expectation value (E) [Real]
- G Cost to open a gap (zero invokes default behaviour) [Integer]
- 5 -E Cost to extend a gap (zero invokes default behaviour) [Integer]
- v Number of one-line descriptions (v) [Integer]
- b Number of alignments to show (b) [Integer]
- i Query File [File In]
- o BLAST report Output File [File Out] Optional

10

The "hits" to one or more database sequences by a queried sequence produced by BLASTN, BLASTP, FASTA, or a similar algorithm, align and identify similar portions of sequences. The hits are arranged in order of the degree of similarity and the length of sequence overlap. Hits to a database sequence generally represent an overlap over only a fraction of the sequence length of the queried sequence.

15

The BLASTN and FASTA algorithms also produce "Expect" or E values for alignments. The E value indicates the number of hits one can "expect" to see over a certain number of contiguous sequences by chance when searching a database of a certain size. The Expect value is used as a significance threshold for determining whether the hit to a database, such as the preferred EMBL database, indicates true similarity. For example, an E value of 0.1 assigned to a hit is interpreted as meaning that in a database of the size of the EMBL database, one might expect to see 0.1 matches over the aligned portion of the sequence with a similar score simply by chance. By this criterion, the aligned and matched portions of the sequences then have a 90% probability of being the same. For sequences having an E value of 0.01 or less over aligned and matched portions, the probability of finding a match by chance in the EMBL database is 1% or less using the BLASTN or FASTA algorithm.

20

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35

According to one embodiment, "variant" polynucleotides, with reference to each of the polynucleotides of the present invention, preferably comprise sequences having the same number or fewer nucleic acids than each of the polynucleotides of the present invention and producing an E value of 0.01 or less when compared to the polynucleotide of the present invention. That is, a variant polynucleotide is any sequence that has at least a 99% probability of being the same as the

polynucleotide of the present invention, measured as having an E value of 0.01 or less using the BLASTN or FASTA algorithms set at the parameters discussed above.

Variant polynucleotide sequences will generally hybridize to the recited
5 polynucleotide sequence under stringent conditions. As used herein, "stringent conditions" refers to prewashing in a solution of 6X SSC, 0.2% SDS; hybridizing at 65°C, 6X SSC, 0.2% SDS overnight; followed by two washes of 30 minutes each in 1X SSC, 0.1% SDS at 65°C and two washes of 30 minutes each in 0.2X SSC, 0.1% SDS at 65°C.

10 The superantigens of the invention together with their fragments and other variants may be generated by synthetic or recombinant means. Synthetic polypeptides having fewer than about 100 amino acids, and generally fewer than about 50 amino acids, may be generated by techniques well known to those of ordinary skill in the
15 art. For example, such peptides may be synthesised using any of the commercially available solid-phase techniques such as the Merryfield solid phase synthesis method, where amino acids are sequentially added to a growing amino acid chain (see Merryfield, J. Am. Chem. Soc 85: 2146-2149 (1963)). Equipment for automative synthesis of peptides is commercially available from suppliers such as Perkin
20 Elmer/Applied Biosystems, Inc. and may be operated according to the manufacturers instructions.

Each superantigen, or a fragment or variant thereof, may also be produced recombinantly by inserting a polynucleotide (usually DNA) sequence that encodes
25 the superantigen into an expression vector and expressing the superantigen in an appropriate host. Any of a variety of expression vectors known to those of ordinary skill in the art may be employed. Expression may be achieved in any appropriate host cell that has been transformed or transfected with an expression vector containing a DNA molecule which encodes the recombinant protein. Suitable host
30 cells includes procaryotes, yeasts and higher eukaryotic cells. Preferably, the host cells employed are *E. coli*, yeasts or a mammalian cell line such as COS or CHO, or an insect cell line, such as SF9, using a baculovirus expression vector. The DNA sequence expressed in this matter may encode the naturally occurring superantigen, fragments of the naturally occurring protein or variants thereof.

DNA sequences encoding the superantigen or fragments may be obtained, for example, by screening an appropriate *S. pyogenes* cDNA or genomic DNA library for DNA sequences that hybridise to degenerate oligonucleotides derived from partial amino acid sequences of the superantigen. Suitable degenerate oligonucleotides
5 may be designed and synthesised by standard techniques and the screen may be performed as described, for example, in Maniatis *et al.* Molecular Cloning - A Laboratory Manual, Cold Spring Harbour Laboratories, Cold Spring Harbour, NY (1989).

10 Identification of these superantigens and of their properties gives rise to a number of useful applications. A first such application is in the genotyping of organisms by reference to their superantigen profile.

An illustration of this is subtyping of strains of *S. pyogenes*.

15

One feature which has been observed is that all clones of *S. pyogenes* so far found to be positive for SMEZ express either SMEZ-1 or SMEZ-2 but not both. Thus they are mutually exclusive, which enables a rapid diagnostic test which tells whether an isolate or a patient sample is either SMEZ-1 +ve or SMEZ-2 +ve. This will assist in
20 the typing of the isolate.

This general diagnostic approach is most simply achieved by providing a set or primers which amplify either all or a subset of superantigen genes and that generate gene specific fragments. This can be modified to provide a simple
25 qualitative ELISA-strip type kit that detects biotin labelled PCR fragments amplified by the specific primers and hybridised to immobilised sequence specific probes. This has usefulness for screening patient tissue samples for the presence of superantigen producing streptococcal strains.

30 Such approaches are well known and well understood by those persons skilled in the art.

Another approach is to provide monoclonal antibodies to detect each of the streptococcal superantigens. An ELISA kit containing such antibodies would allow
35 the screening of large numbers of streptococcal isolates. A kit such as this would be

useful for agencies testing for patterns in streptococcal disease or food poisoning outbreaks.

Another potential diagnostic application of the superantigens of the invention is in the diagnosis of disease, such as Kawasaki Syndrome (KS).

KS is an acute multi-system vasculitis of unknown aetiology. It occurs world-wide but is most prevalent in Japan or in Japanese ancestry. It primarily affects infants and the young up to the age of 16. It is an acute disease that without treatment, can be fatal. Primary clinical manifestations include

- Prolonged fever
- Bilateral non-exudative conjunctivitis
- Induration and erythema of the extremities
- Inflammation of the lips and oropharynx
- Polymorphous skin rash
- Cervical lymphadenopathy
- In 15-25% of cases, coronary arterial lesions develop.

These indications are used as a primary diagnosis of KS.

In Japan and the US, KS has become one of the most common causes of acquired heart disease in children. Treatment involves the immediate intravenous administration of gamma globulin (IVGG) during the acute phase of the disease and this significantly reduces the level of coronary lesions.

There are two clear phases to the disease, an acute phase and a convalescent phase. The acute phase is marked by strong immune activation. Several reports have suggested that superantigens are involved and many attempts have been made to link the disease to infection with superantigen producing strains of *Streptococcus pyogenes*. Features of the acute phase of KS are the expansion of V β 2 and to a lesser extent V β 8 bearing T cells and an increase of DR expression T cells (a hallmark of T cell activation).

Because SMEZ-2 stimulates both V β 2 and V β 8 bearing T cells, testing for SMEZ-2 production is potentially very useful in the diagnosis of KS.

Antibodies to the superantigens for use in applications such as are described above are also provided by this invention. Such antibodies can be polyclonal but will preferably be monoclonal antibodies.

5

Monoclonal antibodies with affinities of 10^{-8} M⁻¹ or preferably 10^{-9} to 10^{-10} M⁻¹ or stronger will typically be made by standard procedures as described, eg. in Harlow & Lane (1988) or Goding (1986). Briefly, appropriate animals will be selected and the desired immunization protocol followed. After the appropriate period of time, the spleens of such animals are excised and individual spleen cells fused, typically, to immortalised myeloma cells under appropriate selection conditions. Thereafter, the cells are clonally separated and the supernatants of each clone tested for their production of an appropriate antibody specific for the desired region of the antigen.

10

Other suitable techniques for preparing antibodies well known in the art involve *in vitro* exposure of lymphocytes to the antigenic polypeptides, or alternatively, to selection of libraries of antibodies in phage or similar vectors.

15

Also, recombinant immunoglobulins may be produced using procedures known in the art (see, for example, US Patent 4,816,567 and Hodgson J. (1991)).

20

The antibodies may be used with or without modification. Frequently, antibodies will be labeled by joining, either covalently or non-covalently, a substance which provides for a detectable signal. A wide variety of labels and conjugation techniques are known and are reported extensively in the literature. Suitable labels include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent agents, chemiluminescent agents, magnetic particles and the like. Patents teaching the use of such labels include US Patents 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241.

25

30

The immunological assay in which the antibodies are employed can involve any convenient format known in the art.

The nucleotide sequence information provided herein may be used to design probes and primers for probing or amplification of parts of the sme-2, spe-g, spe-h and

35

spe-j genes. An oligonucleotide for use in probing or PCR may be about 30 or fewer nucleotides in length. Generally, specific primers are upwards of 14 nucleotides in length. For optimum specificity and cost effectiveness, primers or 16-24 nucleotides in length are preferred. Those skilled in the art are well versed in the design of
5 primers for use in processes such as PCR.

If required, probing can be done with entire polynucleotide sequences provided herein as SEQ ID NOS 1, 3, 5 and 7, optionally carrying revealing labels or reporter molecules.

10

Such probes and primers also form aspects of the present invention.

Probing may employ the standard Southern blotting technique. For instance, DNA may be extracted from cells and digested with different restriction enzymes.
15 Restriction fragments may then be separated by electrophoresis on an agarose gel, before denaturation and transfer to a nitrocellulose filter. Labelled probes may be hybridised to the DNA fragments on the filter and binding determined. DNA for probing may be prepared from RNA preparations from cells. Probing may optionally be done by means of so-called "nucleic acid chips" (see Marshall and Hodgson
20 (1998) *Nature Biotechnology* 16:27-31).

In addition to diagnostic applications, another application of the superantigens is reliant upon their ability to bind to other cells.

25 One of the most important features of superantigens is that they bind a large number of T cell receptor molecules by binding to the V β domain. They are the most potent of all T cell mitogens and are therefore useful to recruit and activate T cells in a relatively non-specific fashion.

30 This ability enables the formation of constructs in which the superantigen (or at least the T-cell binding portion of it) is coupled to a cell-targeting molecule, particularly an antibody, more usually a monoclonal antibody.

When a monoclonal antibody that targets a specific cell surface antigen (such as a
35 tumor specific antigen) is coupled to a superantigen in such a construct, this generates a reagent that on the one hand will bind specifically to the tumor cell, and

on the other hand recruit and selectively active T cells for the purpose of killing the targeted cell.

5 Bi-specific constructs of this type have important applications in therapy (particularly cancer therapy) and again may be prepared by means known to those skilled in art. For example SMEZ-2 may be coupled to a tumor specific monoclonal antibody. The constructs may be incorporated into conventional carriers for pharmaceutically-active proteins.

10 Various aspects of the invention will now be described with reference to the following experimental section, which is included for illustrative purposes.

EXAMPLE

15 SECTION A: SUPERANTIGEN IDENTIFICATION AND CHARACTERISATION

Materials and Methods

Identification of novel SAGs

20 The novel superantigens were identified by searching the *S. pyogenes* M1 genome database at the University of Oklahoma (<http://www.genome.ou.edu/strep.html>) with highly conserved $\beta 5$ and $\alpha 4$ regions of streptococcal and staphylococcal superantigens, using a TBlastN search programme.

25 The open reading frames were defined by translating the DNA sequences around the matching regions and aligning the protein sequences to known superantigens using the computer programmes Gap. Multiple alignments and dendrograms were performed with Lineup and Pileup. The FASTA programme was used for searching the SwissProt (Amos Bairoch, Switzerland) and PIR (Protein Identification Resource,
30 USA) protein databases.

The leader sequences of SPE-G and SPE-H were predicted using the SP Scan programme. All computer programmes are part of the GCG package (version 8).

Cloning of smeZ, smeZ-2, spe-g and spe-h

Fifty nanograms of *S.pyogenes* M1 (ATCC 700294) or *S.pyogenes* 2035 genomic DNA was used as a template to amplify the smeZ DNA fragment and the smeZ-2 DNA fragment, respectively, by PCR using the primers

- 5 smeZ-forward (TGGGATCCTTAGAAGTAGATAATA) and
smeZ-reverse (AAGAATTCTTAGGAGTCAATTC) and Taq Polymerase (Promega). The primers contain a terminal tag with the restriction enzyme recognition sequences BamHI and EcoRI, respectively. The amplified DNA fragment, encoding the mature protein without the leader sequence (Kamezawa et al, 1997 Infect. Immun. 65
10 no9:38281-33) was cloned into a T-tailed pBlueScript SKII vector (Stratagene).

Spe-g and spe-h were cloned in a similar approach, using the primers spe-g-fw (CTGGATCCGATGAAAATTTAAAAGATTTAA) and spe-g-rev (AAGAATTCGGGGGGAGAATAG), and primers spe-h-fw
15 (TTGGATCCAATTCTTATAATACAACC) and spe-h-rev (AAAAGCTTTTAGCTGATTGACAC), respectively.

- The DNA sequences of the subcloned toxin genes were confirmed by the dideoxy chain termination method using a Licor automated DNA sequencer. As the DNA
20 sequences from the genomic database are all unedited raw data, 3 subclones of every cloning experiment were analyzed to ensure that no Taq polymerase related mutations were introduced.

Expression and purification of rSMEZ, rSMEZ-2, rSPE-G and rSPE-H.

- 25 Subcloned smeZ, smeZ-2 and spe-g fragments were cut from pBlueScript SKII vectors, using restriction enzymes BamHI and EcoRI (LifeTech), and cloned into pGEX-2T expression vectors (Pharmacia). Due to an internal EcoRI restriction site within the spe-H gene, the pBlueScript:spe-h subclone was digested with BamHI and HindIII and the spe-h fragment was cloned into a modified pGEX-2T vector that
30 contains a HindIII 3'cloning site.

- Recombinant SMEZ, rSMEZ-2 and rSPE-H were expressed in *E.coli* DH5α cells as glutathione-S-transferase (GST) fusion proteins. Cultures were grown at 37° C and induced for 3-4 h after adding 0.2 mM isopropyl-β-D-thiogalactopyranoside (IPTG).
35 GST - SPE-G fusion protein was expressed in cells grown at 28° C.

The GST fusion proteins were purified on glutathione agarose as described previously (Li et al, 1997) and the mature toxins were cleaved off from GST by trypsin digestion. All recombinant toxins, except rSMEZ, were further purified by
5 two rounds of cation exchange chromatography using carboxy methyl sepharose (Pharmacia). The GST-SMEZ fusion protein was trypsin digested on the GSH-column and the flow through containing the SMEZ was collected.

Gel electrophoresis

10 All purified recombinant toxins were tested on a 12% SDS-polyacrylamide gel according the procedure of Laemmli. The isoelectric point of the recombinant toxins was determined by isoelectric focusing on a 5.5% polyacrylamide gel using ampholine pH 5-8 (Pharmacia Biotech). The gel was run for 90 min at 1 W constant power.

15

Toxin proliferation assay

Human peripheral blood lymphocytes (PBL) were purified from blood of a healthy donor by Histopaque Ficoll (Sigma) fractionation. The PBL were incubated in 96-well round bottom microtiter plates at 10^5 cells per well with RPMI-10 (RPMI with 10%
20 fetal calf serum) containing varying dilutions of recombinant toxins. The dilution series was performed in 1:5 steps from a starting concentration of 10 ng/ml of toxin. Pipette tips were changed after each dilution step. After 3 days 0.1 μ Ci [3 H]thymidine was added to each well and cells were incubated for another 24 h. Cells were harvested and counted on a scintillation counter.

25

Mouse leukocytes were obtained from spleens of 5 different mouse strains (SJL, B10.M, B10/J, C3H and BALB/C). Splenocytes were washed in DMEM-10, counted in 5% acetic acid and incubated on microtiter plates at 10^5 cells per well with DMEM-10 and toxins as described for human PBLs.

30 TcR V β analysis.

V β enrichment analysis was performed by anchored multiprimer amplification (Hudson et al, 1993, J exp Med 177:175-185). Human PBLs were incubated with 20 pg/ml of recombinant toxin at 10^6 cells/ml for 3 d. A two-fold volume expansion of
35 the culture followed with medium containing 20 ng/ml IL-2. After another 24h,

stimulated and resting cells were harvested and RNA was prepared using Trizol reagent (Life Tech). A 500 bp β -chain DNA probe was obtained by anchored multiprimer PCR as described previously (38), radiolabeled and hybridized to del (36) individual V β s and a C β DNA region dot blotted on a Nylon membrane. The
5 membrane was analysed on a Molecular Dynamics Storm Phosphor imager using ImageQuant software. Individual V β s were expressed as a percentage of all the V β s determined by hybridization to the C β probe.

Jurkat cell assay

10 Jurkat cells (a human T cell line) and LG-2 cells (a human B lymphoblastoid cell line, homozygous for HLA-DR1) were harvested in log phase and resuspended in RPMI-10. One hundred microliter of the cell suspension, containing 1×10^5 Jurkat cells and 2×10^4 LG-2 cells were mixed with 100 μ l of varying dilutions of recombinant toxins on 96 well plates. After incubating overnight at 37° C, 100 μ l
15 aliquots were transferred onto a fresh plate and 100 μ l (1×10^4) of SeI cells (IL-2 dependent murine T cell line) per well were added. After incubating for 24 h, 0.1 μ Ci [3 H]thymidine was added to each well and cells were incubated for another 24 h. Cells were harvested and counted on a scintillation counter. As a control, a dilution series of IL-2 was incubated with SeI cells.

20

Computer aided modelling of protein structures

Protein structures of SMEZ-2, SPE-G and SPE-H were created on a Silicon Graphics computer using InsightII/Homology software. The superantigens SEA, SEB and SPE-C were used as reference proteins to determine structurally conserved regions
25 (SCRs). Coordinate files for SEA (1ESF), for SEB (1SEB) and for SPE-C (1AN8) were downloaded from the Brookhaven Protein Database. The primary amino acid sequences of the reference proteins and SMEZ-2, SPE-G and SPE-H, respectively, were aligned and coordinates from superimposed SCR's were assigned to the model proteins. The loop regions between the SCRs were generated by random choice.
30 MolScript software (PJ Kraulis, 1991, J App Crystallography 24:946-50) was used for displaying the computer generated images.

Radiolabeling and LG-2 binding experiments

Recombinant toxin was radioiodinated by the chloramine T method as previously
35 described (by Li et al. 1997). Labeled toxin was separated from free iodine by size

exclusion chromatography using Sephadex G25 (Pharmacia). LG2 cells were used for cell binding experiments, as described (Li et al. 1997). Briefly, cells were harvested, resuspended in RPMI-10 and mixed at 10^6 cells/ml with ^{125}I -tracer toxin (1 ng) and 0.0001 to 10 μg of unlabeled toxin and incubated at 37°C for 1 h. After
5 washing with ice cold RPMI-1 the pelleted cells were analyzed in a gamma counter. For zinc binding assays the toxins were incubated in either RPMI-10 alone, in RPMI-10 with 1 mM EDTA or in RPMI-10 with 1mM EDTA, 2 mM ZnCl_2 .

Scatchard analysis was performed as described by Cunningham et al. (1989). For
10 competitive binding studies, 1 ng of ^{125}I -tracer toxin (rSMEZ, rSMES-2, rSPE-G, rSPE-H, rSEA, rSPE-C, or rTSST) was incubated with 0.0001 to 10 μg of unlabeled toxin (rSMEZ, rSMES-2, rSPE-G, rSPE-H, rSEA, rSEB, rSPE-C, and rTSST) for 1h. For SEB inhibition studies, 20 ng of ^{125}I -rSEB was used as tracer and samples were incubated for 4h.

15

Results

Identification and sequence analysis of superantigens.

The Oklahoma University *Streptococcus pyogenes* M1 genome database is accessible
20 via the internet and contains a collection of more than 300 DNA sequence contigs derived from a shot gun plasmid library of the complete *S. pyogenes* M1 genome. The currently available DNA sequences cover about 95% of the total genome. This database was searched with a highly conserved superantigen peptide sequence, using a search program that screens the DNA database for peptide sequences in all
25 6 possible reading frames. 8 significant matches and predicted the open reading frames (ORFs) were found by aligning translated DNA sequences to complete protein sequences of known SAgS.

Five matches gave complete ORFs with significant homology to streptococcal and
30 staphylococcal superantigens. Three of these ORFs correlate to SPE-C, SSA and the recently described SMEZ (Kamezawa et al. 1997), respectively. The remaining two ORFs could not be correlated to any known protein in the SwissProt and PIR databases. These novel putative superantigen genes were named spe-g and spe-h (see Figs 3 and 4). One ORF could not be generated completely due to its location
35 close to the end of a contig. The DNA sequence of the missing 5'-end is located on

another contig, and individual contigs have yet to be assembled in the database. However, the available sequence shows an ORF for the 137 COOH-terminal amino acid residues of a putative novel superantigen which could not be found in the existing protein databases. This gene was named spe-j (see Fig. 5).

5

In two cases a complete ORF could not be defined due to several out-of-frame mutations. Although DNA sequencing errors on the unedited DNA sequences cannot be completely ruled out, the high frequency of inserts and deletions probably represent natural mutation events on pseudogenes, which are no longer used.

10

To produce recombinant proteins of SMEZ, SPE-G and SPE-H, individual genes (coding for the mature toxins without leader sequence) were amplified by PCR, and subcloned for DNA sequencing. Both, *Str. pyogenes* M1 and *Str. pyogenes* 2035 genomic DNA were used and individual toxin gene sequences compared between the two strains. The spe-h gene was isolated from M1 strain, but could not be amplified from strain 2035 genomic DNA suggesting a restricted strain specificity for this toxin. The spe-g gene was cloned from both M1 and 2035, and DNA sequence analysis of both genes showed no differences. The full length smeZ gene was isolated from both strains, but DNA sequence comparison revealed some striking differences. The smeZ gene of strain 2035 showed nucleotide changes in 36 positions (or 5%) compared to smeZ from strain M1 (Fig. 1). The deduced protein sequences differed in 17 amino acid residues (or 8.1%). This difference was sufficient to indicate a new gene. This gene was named smeZ-2, because it is 95% homologous to smeZ (see Fig. 2).

25

The most significant difference between SMEZ and SMEZ-2 is an exchanged pentapeptide sequence at position 96-100, where the EEPMS sequence of SMEZ is converted to KTSIL in SMEZ2 (Fig. 1). A second cluster is at position 111-112, where an RR dipeptide is exchanged for GK in SMEZ-2. The remaining 10 different residues are spread over almost the entire primary sequence.

30

A revised superantigen family tree, based on primary amino acid sequence homology now shows 3 general subfamilies; group A comprises SPE-C, SPE-J, SPE-G, SMEZ and SMEZ-2, group B comprises SEC1-3, SEB, SSA, SPE-A and SEG and

group C comprises SEA, SEE, SED, SEH and SEI. Two superantigens, TSST and SPE-H do not belong to any one of those subfamilies.

5 SMEZ, SMEZ-2, SPE-G and SPE-J are most closely related to SPE-C, increasing the number of this subfamily from 2 to 5 members. SPE-G shows the highest protein sequence homology with SPE-C (38.4% identity and 46.6% similarity). The homology of SPE-J to SPE-C is even more significant (56% identity and 62% similarity), but this comparison is only preliminary due to the missing NH₂-terminal sequence. SMEZ shows 30.9% / 40.7% homology to SPE-C and SMEZ-2 is 92% /
10 93% homologous to SMEZ.

SPE-H builds a new branch in the family tree and is most closely related to SED, showing 25% identity and 37.3% similarity.

15 Multiple alignment of SAg protein sequences (Fig. 1) shows that similarities are clustered within structure determining regions, represented by α 4, α 5, β 4 and β 5 regions. This applies to all toxins of the superantigen family (data not shown) and explains why superantigens like SPE-C and SEA have very similar overall structures despite their rather low sequence identity of 24.4 %.

20

Although SPE-H is less related to SPE-C it shows 2 common features with the "SPE-C subfamily": (I) a truncated NH₂-terminus, lacking the α 1 region and (II) a primary zinc binding motif (H-X-D) at the C-terminus (Fig. 1). It has been shown for several superantigens that this motif is involved in a zinc coordinated binding to the β -chain
25 of HLA-DR1.

Fusion proteins of GST-SMEZ, GST-SMEZ-2 and GST-SPE-H were completely soluble and gave yields of about 30 mg per liter. The GST-SPE-G fusion was insoluble when grown at 37° C, but mostly soluble when expressed in cells growing
30 at 28° C. Although soluble GST-SPE-G yields were 20-30 mg per liter, solubility decreased after cleavage of the fusion protein with trypsin. Soluble rSPE-G was achieved by diluting the GST-SPE-G to less than 0.2 mg/ml prior to cleavage. After cation exchange chromatography, purified rSPE-G could be stored at about 0.4 mg/ml.

35

Recombinant SMEZ could not be separated from GST by ion exchange chromatography. Isoelectric focusing revealed that the isoelectric points of the two proteins are too similar to allow separation (data not shown). Therefore, rSMEZ was released from GST by cleaving with trypsin while still bound to the GSH agarose column. Recombinant SMEZ was collected with the flow through.

The purified recombinant toxins were applied to SDS-PAGE and isoelectric focusing (Fig. 6). Each toxin ran as a single band on the SDS PAA gel confirming their purity and their calculated molecular weights of 24.33 (SMEZ), 24.15 (SMEZ-2), 24.63 (SPE-G) and 23.63 (SPE-H) (Fig. 6A). The isoelectric focusing gel (Fig. 6B) shows a significant difference between rSMEZ and rSMEZ-2. Like most other staphylococcal and streptococcal toxins, rSMEZ-2 possesses a slightly basic isoelectric point at pH 7-8, but rSMEZ is acidic with an IEP at pH 6-6.5.

15 *T cell proliferation and V β specificity*

To ensure the native conformation of the purified recombinant toxins, a standard [³H]thymidine incorporation assay was performed to test for their potency to stimulate peripheral blood lymphocytes (PBLs). All toxins were active on human T cells (Fig. 7). Recombinant SEA, rSEB, rSPE-C and rTSST were included as reference proteins. The mitogenic potency of these toxins was lower than described previously, but is regarded as a more accurate figure. In previous studies, a higher starting concentration of toxin (100 ng/ml) was used and tips were not changed in between dilutions. This led to significant carryover across the whole dilution range. On this occasion, the starting concentration was 10 ng/ml and tips were changed in between dilutions preventing any carryover.

The half maximal response for rSPE-G and rSPE-H was 2 pg/ml and 50 pg/ml, respectively. No activity was detected at less than 0.02 pg/ml and 0.1 pg/ml, respectively. Both toxins are therefore less potent than rSPE-C. Recombinant SMEZ was similar in potency to rSPE-C, with a P_{50%} value of 0.08 pg/ml and no detectable proliferation at less than 0.5 fg/ml. Recombinant SMEZ-2 showed the strongest mitogenic potency of all toxins tested or, as far as can be determined, described elsewhere. The P_{50%} value was determined at 0.02 pg/ml and rSMEZ-2 was still active at less than 0.1 pg/ml. All P_{50%} values are summarized in Table 1.

TABLE 1

POTENCY OF RECOMBINANT TOXINS ON HUMAN AND MOUSE T CELLS.						
PROLIFERATION POTENTIAL P _{50%} [pg/ml]						
TOXIN	HUMAN	SJL	B10.M	B10/J	C3H	BALB/C
SEA	0.1	20	12	1.8	19	1000
SEE	0.2	10	12	1.5	50	15
SEB	0.8	7000	80,000	5000	10,000	1000
TSST	0.2	20	1000	1.2	100	10
SPE-C	0.1	>100,000	>100,000	>100,000	>100,000	>100,000
SMEZ	0.08	80	80	100	9000	200
SMEZ-2	0.02	100	15	10	800	18
SPE-G	2	>100,000	>100,000	>100,000	>100,000	>100,000
SPE-H	50	15	800	5000	100	1000

5

Human PBLs and mouse T cells were stimulated with varying amounts of recombinant toxin. The P_{50%} value reflects the concentration of recombinant toxin required to induce 50% maximal cell proliferation. No proliferation was detected for rSPE-C and rSPE-G at any concentration tested on murine T cells.

Murine T cells from 5 different mouse strains were tested for their mitogenic response to rSMEZ, rSMEZ-2, rSPE-G and rSPE-H (Table 1). Recombinant SPE-G showed no activity against any of the mouse strains tested. Recombinant SPE-H, rSMEZ and rSMEZ-2 showed varied potency depending on the individual mouse strain. For example, rSMEZ-2 was 500-fold more potent than rSPE-H in the B10/J strain, while rSPE-H was 7.5-fold more active than rSMEZ-2 in the SJL strain.

The most consistently potent toxin on murine T cells was rSMEZ-2 with $P_{50\%}$ values of 10 pg/ml in B10/J and 800 pg/ml in C3H. Recombinant SMEZ varied between 80 pg/ml in SJL and B10.M and 9000 pg/ml in C3H. The $P_{50\%}$ value for rSPE-H was between 15 pg/ml in SJL and 5000 pg/ml in B10/J.

TABLE 2

V β SPECIFICITY OF RECOMBINANT TOXINS ON HUMAN PBLs.					
PERCENT V β ENRICHMENT					
V β	Resting	SMEZ	SMEZ-2	SPE-G	SPE-H
1.1	0.2	0.3	0.4	1.2	1
2.1	0.4	<u>8.4</u>	1	<u>17.9</u>	<u>8.6</u>
3.2	4.8	3.1	2.5	3	2.4
4.1	3.5	<u>24.8</u>	<u>14.4</u>	<u>11.2</u>	5.2
5.1	6.2	1.4	2.5	5.7	2.2
5.3	5.6	2.2	4.1	4.7	4.1
6.3	3	0.8	2.3	4.7	3.5
6.4	5.4	2.1	5.9	9.6	5.6
6.9	6.9	3.5	9.3	<u>19.1</u>	12.2
7.3	3.5	<u>15.3</u>	7.3	3.2	<u>12.6</u>
7.4	9	13.5	11.7	2.9	6.3
8.1	8.7	<u>20.7</u>	<u>36</u>	4.5	2.4
9.1	0.3	0.05	0	<u>1.2</u>	<u>2.3</u>
12.3	0.8	1.6	2	<u>3.2</u>	2.6
12.5	3	1.2	2	3	2.3
15.1	0.6	0.5	0.7	1.2	0.8
23.1	0.2	0.1	0.3	0.8	<u>1</u>
total	62.1	99.7	102.8	97.1	75.2

Human PBLs were incubated with 20 pg/ml of recombinant toxin for 4d. Relative enrichment of V β cDNAs was analyzed from RNA of stimulated and resting PBLs by anchored primer PCR and reverse dot blot to a panel of 17 different V β cDNAs.

5

The values representing the highest V β enrichment are underlined.

The human TcR V β specificity of the recombinant toxins was determined by multiprimer anchored PCR and dot blot analysis using a panel of 17 human V β DNA regions. The V β enrichment after stimulation with toxin was compared to the V β profile of unstimulated PBLs (Table 2). The sum total of all V β s stimulated by rSMEZ, rSMEZ-2 and rSPE-G was close to 100 % suggesting that the V β s used in the panel represent all the targeted V β s. On the other hand, the total of the V β s stimulated by rSPE-H was only 75%. It is therefore likely that rSPE-H also stimulated some less common V β s, which are not represented in the panel. The most dramatic response was seen with all toxins, except rSMEZ2, on V β 2.1 bearing T cells (21-fold for rSMEZ, 45-fold for rSPE-G and 22-fold for rSPE-H). In contrast, rSMEZ2 gave only a 2.5-fold increase of V β 2.1 T-cells. SPE-G also targeted V β 4.1, V β 6.9, V β 9.1 and V β 12.3 (3-4 fold). A moderate enrichment of V β 12.6, V β 9.1 and V β 23.1 (4-8 fold) was observed with rSPE-H. Both, rSMEZ and rSMEZ2, targeted V β 4.1 and V β 8.1 with similar efficiency (3-7-fold). This finding is of particular interest, because V β 8.1 activity had been found in some, but not all *Str. pyogenes* culture supernatants and in crude preparations of SPE-A and SPE-C. Moreover, SPE-B has often been claimed to have V β 8 specific activity, but has since been shown to be a contaminant previously called SpeX. The ability of rSMEZ and rSMEZ-2 to stimulate the V β 8.1 Jurkat cell line was tested (Fig. 8) Recombinant SMEZ was less potent than the control toxin (rSEE), showing a half maximal response of 0.2 ng/ml, compared to 0.08 ng/ml with rSEE, but rSMEZ-2 was more potent than rSEE (0.02 ng/ml). No proliferation activity was observed with the negative control toxin rSEA.

25 *MHC class II binding*

To determine if there were significant structural differences, the protein structures of SMEZ-2, SPE-G and SPE-H were modelled onto the superimposed structurally conserved regions of SEA, SEB and SPE-C. The models showed that in all three proteins, the 2 amino acid side chains of the COOH-terminal primary zinc binding motif are in close proximity to a third potential zinc ligand to build a zinc binding site, similar to the zinc binding site observed in SEA and SPE-C.

The zinc binding residues in SPE-C are H167, H201, D203, and it is thought that H81 from the HLA-DR1 β -chain binds to the same zinc cation to form a regular tetrahedral complex. The two ligands of the primary zinc binding motif, H201 and

D203, are located on the β 12 strand, which is part of the β -grasp motif, a common structural domain of superantigens. The third ligand, H167, comes from the β 10 strand (Roussel et al. 1997).

- 5 In the model of SPE-G three potential zinc binding ligands (H167, H202 and D204) are located at corresponding positions. In the SMEZ-2 and the SPE-H models, the two corresponding β 12 residues are H202, D204 and H198, D200, respectively. The third ligand in SPE-H (D160) and in SMEZ-2 (H162) comes from the β 9 strand and is most similar to H187 in SEA. It has been shown from crystal structures that H167
10 of SPE-C and H187 of SEA are spatially and geometrically equivalent sites (Scad et al. 1997, Embo J 14 no 14:3292-301; Roussel et al. 1997).

- All superantigens examined so far, except SPE-C, bind to a conserved motif in the MHC class II α 1-domain. In SEB and TSST, hydrophobic residues on the loop
15 between the β 1 and β 2 strand project into a hydrophobic depression in the MHCII α 1-domain. This loop region has changed its character in SPE-C, where the hydrophobic residues (F44, L45, Y46 and F47 in SEB) are substituted by the less hydrophobic residues T33, T34 and H35. A comparison of this region on the computer generated models revealed that the generic HLA-DR1 α -chain binding site
20 might also be missing. As the loop regions are generated by random choice, no conclusions can be drawn from their conformation in the models. However, in none of the three models does the β 1- β 2-loop have the required hydrophobic features observed in SEB and TSST Swaminathan, S. *et al.*, *Nature* 359, No. 6398:801-6 (1992), Acharya *et al.*, *Nature* 367, No. 6458: 94-7 (1994). The residues are I25, D26,
25 F27, K28, T29 and S30 in SMEZ-2, T31, T32, N33, S34 in SPE-G and K28, N29, S30, P31, D32, I33, V34 and T35 in SPE-H.

- SMEZ-2 differs from SMEZ in only 17 amino acids. In the model of SMEZ-2 with the position of those 17 residues, most of the exchanges are located on loop regions,
30 most significantly on the β 5- β 6 loop with 5 consecutive residues replaced. The potential zinc binding site and the β 1- β 2 loop are not affected by the replaced amino acids.

- The TcR V β specificity differs between SMEZ and SMEZ-2 by one V β . SMEZ strongly
35 stimulates V β 2 T cells, but SMEZ-2 does not (Table 2). One or more of the 17

exchanged residues in SMEZ/SMEZ-2 may therefore be directly involved in TcR binding. The exact position of the TcR binding site can not be predicted from the model as several regions have been implicated in TcR binding for different toxins. Crystal structures of SEC2 and SEC3, complexed with a TcR β -chain indicated the
5 direct role of several residues located on α 2, the β 2- β 3 loop, the β 4- β 5 loop and α 4 (Fields et al. 1996 Nature 384 no 6605:188-92). On the other hand, binding of TSST to the TcR involves residues from α 4, the β 7- β 8 loop and the α 4- β 9 loop (Acharya et al. 1994, Nature 367 no 6548:94-7). The SMEZ-2 model shows 3 residues, which may contribute to TcR binding. In SMEZ, Lys is exchanged for Glu at position 80
10 and Thr is exchanged for Ile at position 84, both on the β 4- β 5 loop. On the COOH-terminal end of the α 4 helix, Ala is replaced by Ser at position 143.

The results from the computer modelled protein structures suggest that all 4 toxins, SMEZ, SMEZ-2, SPE-G and SPE-H, might bind to the HLA-DR1 β -chain in a zinc
15 dependent fashion, similar to SEA and SPE-C, but might not be able to interact with the HLA-DR1 α -site, a situation that has so far only been observed with SPE-C (Roussel et al. 1997; Li et al. 1997).

To find out whether or not zinc is required for binding of the toxins to MHC class II,
20 a binding assay was performed using human LG-2 cells (which are MHC class II expressing cells homozygous for HLA-DR1). Direct binding of ^{125}I -labeled toxins was completely abolished in the presence of 1 mM EDTA (Fig. 9, Table 3). When 2 mM ZnCl_2 was added, binding to the LG-2 cells could be restored completely. These results show that the toxins bind in a zinc dependent mode, most likely to the HLA-
25 DR1 β -chain similar to SEA and SPE-C. However, it does yet not exclude the possibility of an additional binding to the HLA-DR1 α -chain.

TABLE 3

BINDING AFFINITIES AND ZINC DEPENDENCIES FOR SUPERANTIGENS TO HUMAN CLASS II		
TOXIN	MHC CLASS II BINDING kd [nM]	ZINC DEPENDENCY
SEA	36/1000	++
SEB	340	-
TSST	130	-
SPE-C	70	++
SMEZ	65/1000	++
SMEZ-2	25/1000	++
SPE-G	16/1000	++
SPE-H	37/2000	++

The binding affinities of the toxins to MHC class II were determined by Scatchard analysis using LG-2 cells. Zinc dependency was determined by binding of recombinant toxins to LG-2 cells in the presence and absence of EDTA, as described in the Materials and Methods section.

The biphasic binding of SEA to HLA-DR1 can be deduced from Scatchard analysis. It shows that SEA possesses a high affinity binding site of 36 nM (which is the zinc dependent β -chain binding site) and a low affinity binding site of 1 μ M (α -chain binding site). On the other hand, only one binding site for HLA-DR1 was deduced from Scatchard analysis with SEB, TSST and SPE-C, respectively (Table 3).

Therefore, Scatchard analysis was performed with radiolabeled rSMEZ, rSMEZ-2, rSPE-G and rSPE-H using LG-2 cells. All four toxins showed multiphasic curves with at least 2 binding sites on LG-2 cells, a high affinity site of 15-65 nM and a low affinity site of 1-2 μ M (Fig. 10, Table 3).

In a further attempt to determine the orientation of the toxins on MHC class II competition binding experiments were performed. The recombinant toxins and reference toxins (rSEA, rSEB, rSPE-C and rTSST) were radiolabeled and tested with excess of unlabeled toxin for binding to LG-2 cells. The results are summarized in Fig. 11. Both, rSEA and rSPE-C, inhibited binding of labeled rSMEZ, rSMEZ-2, rSPE-G and rSPE-H, respectively. However, rSPE-C only partially inhibited binding (50%) of the labeled rSMEZ-2 (Fig. 12). Recombinant SEB did not compete with any other toxin, even at the highest concentration tested. Recombinant TSST was only slightly competitive against ^{125}I -labeled rSMEZ, rSMEZ-2 and rSPE-G, respectively, and did not inhibit rSPE-H binding at all.

Reciprocal competition experiments were performed. Recombinant SMEZ, rSMEZ-2 and rSPE-H prevented ^{125}I -rSEA from binding to LG-2 cells. However, only partial competition (50%) was observed even at the highest toxin concentrations (10,000 fold molar excess). Recombinant SPE-G did not prevent binding of ^{125}I -rSEA and ^{125}I -rTSST binding was only partially inhibited by rSMEZ, rSMEZ-2 and rSPE-H, but not by rSPE-G. Significantly, none of the toxins inhibited ^{125}I -rSEB binding, even at the highest concentration tested.

In a further set of competition binding experiments, rSMEZ, rSMEZ-2, rSPE-G and rSPE-H were tested for competition against each other. Both, rSMEZ and rSMEZ-2 competed equally with each other and also prevented binding of labeled rSPE-G and rSPE-H. In contrast, rSPE-G and rSPE-H did not inhibit any other toxin binding suggesting that these toxins had the most restricted subset of MHC class II molecules, which represent specific receptors.

SECTION B: GENOTYPING

Genotyping of *S.pyogenes* isolates

Purified genomic DNA from all *Str. Pyogenes* isolates was used for PCR with specific primers for the smeZ, spe-g and spe-h genes as described above and by Proft (1999). In addition, a primer pair specific to a DNA region encoding the 23S rRNA, oligo 23rRNA forward (GCTATTTTCGGAGAGAACCAG) and oligo 23rRNA reverse (CTGAAACATCTAAGTAGCTG) was designed and used for PCR as a positive control.

Southern blot analysis

About 5µg of genomic DNA was digested using restriction enzyme HindIII (GIBCO) and loaded onto a 0.7% agarose gel. The DNA was transferred from the gel to a
5 Hybond-N+ nylon membrane (Amersham) as described by Maniatis (1989). A 640 bp DNA fragment of the smeZ-2 gene was radiolabeled using the RadPrime Labeling System (GIBCO) and α ³²P-dCTP (NEN). The nylon blots were hybridized with the radiolabeled probe in 2x SSC, 0.5% SDS, 5x Denhards overnight at 65°C. After washing twice in 0.2x SSC, 0.1% SDS at 65°C the blots were analysed on a Storm
10 PhosphorImager.

RESULTS

PCR based genotyping was performed in order to determine the frequency of the
15 genes smeZ, spe-g and spe-h in streptococcal isolates (Table 4). The PCR primers for smeZ were designed to anneal with both genes, smeZ and smeZ-2. 103 isolates were collected between 1976 and 1998 from varying sites in patients with varying infections, although the majority were from sore throats. They comprised 94 group A *Streptococcus* (GAS) and 9 non-GAS, which were *S. agalactiae* (group B), *S. equis*
20 (group C) and *Streptococcus spp* (group C). There are 25 distinct M/emm types represented among the GAS isolates, 13 isolates are M non-typable (MNT) and in 2 cases the M type is unknown. The analysis was undertaken blinded to the details of each isolate and 2 duplicate isolates were included (95/31 and 4202) to demonstrate the reproducibility of the testing procedure. The isolates are listed in 2
25 groups. Group 1 contained isolates collected within a large time frame (1976 to 1996). Group 2 comprised of isolates collected within a short time (1998).

All of the 9 non-GAS isolates (belonging to groups B, C and G) were negative for the tested sag genes. The frequencies for smeZ, spe-g and spe-h within the GAS isolates
30 were 95.6%, 100% and 23.9% respectively. A correlation between a certain M/emm type and the presence of the spe-h gene could not be established. The deficiency in this current set was that only 5M/emm types were represented by more than one isolate. The most frequent serotype was M/emm 12 with 13 isolates, from which 7 were positive and 6 were negative for spe-h suggesting genetic diversity within the

M/emm12 strain. In contrast, all 12 tested NZ1437/M89 isolates were negative for spe-h.

5 The high frequencies of smeZ and spe-g is of particular interest as this has not been described for any other streptococcal sag gene thus far. Other spe genes, like speA, speC and ssa are found at much lower frequencies and horizontal gene transfer might explain the varying frequencies of these genes in different strains. In contrast, both smeZ and spe-g were found in virtually all tested GAS isolates. Only 4 GAS isolates (11152, 11070, 94/229 and 11610) tested negative for smeZ. These 10 were PT2612, emm65, M49 and emm57. Southern hybridisation was performed to find out if the negative PCR results were due to lack of the smeZ gene or to lack/alteration of the primer binding site(s). HindIII digested genomic DNA of selected streptococcal isolates was probed with a 640 bp radiolabeled smz-2 PCR fragment (Fig. 13). The smeZ gene is located on a 1953 bp HindIII fragment of about 15 4kb (fragment B), but not to the SMEZ bearing fragment A (lanes 4, 6, 9, 10). In addition, the smeZ probe bound to a second DNA fragment of about 4.2 kb (fragment C) in isolate 11152 (lane 4). In the M1 reference strain (lane 1) and in isolate 4202 (lane 8) the smeZ probe also bound to fragment B, in addition to fragment A. Fragment B in the M1 strain contains a 180 bp region that shares 97% sequence 20 homology with the 3' end of the smeZ gene. These results suggest that the 4 PCR negative isolates possess a truncated smeZ gene or a smeZ-like sequence, but not a complete smeZ gene.

Tabl 4

Group 1: Isolates collected between 1976 and 1996									
Strain No.	Group	M/emm	Site	Disease	Rib.DNA	Spe-g	Spe-h	Smez	Vp8
FP 1943	A	M53	ts	ST	+	+	-	+	-
FP 2658	A	M59	ts	ST	+	+	-	+	-
FP 4223	A	M80	ts	ST	+	+	-	+	+
FP 5417	A	M41	ts	ST	+	+	-	+	+
FP 5847	A	M1	ts	ST	+	+	-	+	+
FP 5971	A	M57	ts	ST	+	+	+	+	-
1/5045	A	M4	ts	ST	+	+	-	+	+
79/1575	A	M1	ts	Tcarriage	+	+	+	+	+
81/3033	A	M12	ts	ST	+	+	+	+	+
82/20	A	M4	sk	ulcer	+	+	-	+	+
82/532	A	M12	ts	ST	+	+	+	+	+
82/675	A	NZ1437 §	ws	wound	+	+	-	+	+
84/141	A	M12	ts	ST	+	+	+	+	+
84/1733	A	M4	ts	ST	+	+	-	+	+
84/781	A	NZ1437 §	ts	ST	+	+	-	+	+
85/1	A	M12	ts	ST	+	+	-	+	+
85/167	A	M12	ts	ST	+	+	+	+	+
85/314	A	NZ1437 §	ws	wound	+	+	-	+	+
85/437	A	M81	ws	inf eczema	+	+	-	+	+
85/722	A	n.d.	?	?	+	+	-	+	-
86/435	A	M4	ts	RF	+	+	-	+	+
87/169	A	M12	ts	ST	+	+	+	+	+
87/19	A	M12	ts	ST	+	+	+	+	+
87/781	A	M12	ts	ST	+	+	-	+	+
88/627	A	M12	sk	wound	+	+	-	+	-
89/22	A	M12	ts	fever	+	+	-	+	+
89/25	A	M12	ur	erysipelas	+	+	+	+	+
89/26	A	M1	ts	AGN	+	+	-	+	+
89/54	A	NZ1437 §	ts	ST	+	+	-	+	+
90/306	A	M5	ear	otorrhoea	+	+	-	+	+
90/424	A	M4	ts	ST	+	+	-	+	+
91/542	A	M12	ts	ST	+	+	-	+	+
94/11	A	NZ1437 §	ps	abscess	+	+	-	+	+
94/229	A	M49	hvs	endometr.	+	+	+	-	-
94/330	A	M4	ts	SF	+	+	-	+	+
94/354	A	M12	ts	ST	+	+	-	+	+
94/384	A	M4	sk	wound	+	+	-	+	+
94/712	A	NZ1437 §	ws	cellulitis	+	+	-	+	+
95/127	A	NZ1437 §	bc	cellulitis	+	+	-	+	+

95/31	A	NZ1437 §	ws	abscess	+	+	-	+	+
95/31(2)	A	NZ1437 §	ws	abscess	+	+	-	+	+
95/361	A	NZ1437 §	ps	abscess	+	+	-	+	+
96/1	A	n.d.	?	?	+	+	-	+	+
96/364	A	NZ1437 §	be	burns	+	+	-	+	+
96/551	A	M4	eye	eye infect	+	+	-	+	+
96/610	A	M4	ts	SF	+	+	-	+	+
D21	A	M1	ts	Tcarriage	+	+	-	+	+
RC4063	C	-	ts	ST	+	-	-	-	-
SP9205	C	-	ts	ST	+	-	-	-	-
NI6174	G	-	ts	ST	+	-	-	-	-
NI6192	B	-	ts	ST	+	-	-	-	-
VC4141	G	-	ts	ST	+	-	-	-	-

Group 2: Isolates collected in 1998										
Strain No.	student ID	group	M/emm	site	disease	rib.DNA	spe-g	spe-h	smez	Vp8
4202 *	3310	A	NZ5118Π	ts	ST	+	+	-	+	+
4202(2)	3310	A	NZ5118Π	ts	ST	+	+	-	+	+
9606	2252	A	MNT	ts	ST	+	+	-	+	-
9639	2184	A	MNT	ts	ST	+	+	+	+	+
9779	3230	A	emm56	ts	ST	+	+	-	+	+
9893	6144	A	PT180	ts	ST	+	+	+	+	+
9894	6564	A	emm59	ts	ST	+	+	-	+	+
10019	6264	A	emm44	ts	ST	+	+	+	+	-
10028	9366	A	emm41	ts	ST	+	+	-	+	+
10134	1880	A	ST4547	ts	ST	+	+	-	+	-
10303	3564	A	emm59	ts	ST	+	+	-	+	-
10307	4850	A	NZ5118Π	ts	ST	+	+	-	+	+
10438	4904	A	ST3018	ts	ST	+	+	-	+	+
10463	TSP	A	emm49	ts	ST	+	+	-	+	-
10649	11510	A	ST2267	ts	ST	+	+	-	+	+
10730	11503	A	MNT	ts	ST	+	+	-	+	-
10742	3374	A	ST809	ts	ST	+	+	-	+	+
10761	3254	A	MNT	ts	ST	+	+	-	+	-
10763	6614	PT 3875	ts	ST	+	+	-	+	-	1078 2
4850	A	MNT	ts	ST	+	+	+	+	+	+
10791	10290	A	MNT	ts	ST	+	+	+	+	+
10792	10308	A	MNT	ts	ST	+	+	+	+	-
10846	8854	A	NZ1437 §	ts	ST	+	+	-	+	+

10902	6264	A	NZ5118Π	ts	ST	+	+	-	+	+
10989	5194	A	PT2841	ts	ST	+	+	-	+	-
11070	1434	A	emm65	ts	ST	+	+	+	-	-
11072	1880	A	ST4547	ts	ST	+	+	-	+	-
11083	4538	A	MNT	ts	ST	+	+	-	+	-
11093	9791	A	MNT	ts	ST	+	+	+	+	+
11152	2030	A	PT2612	ts	ST	+	+	+	-	-
11222	4928	A	NZ5118Π	ts	ST	+	+	+	+	+
11227	8854	A	emm14	ts	ST	+	+	-	+	-
11244	2252	A	ST4547	ts	ST	+	+	-	+	-
11276	4524	A	MNT	ts	ST	+	+	-	+	-
11299	2950	A	emm80	ts	ST	+	+	-	+	+
11574	3186	A	ST809	ts	ST	+	+	-	+	+
11580	3280	A	emm53	ts	ST	+	+	-	+	-
11610	2424	A	emm57	ts	ST	+	+	+	-	-
11646	1880	A	ST4547	ts	ST	+	+	-	+	-
11681	3564	A	emm12	ts	ST	+	+	-	+	+
11686	5528	A	PT5757	ts	ST	+	+	-	+	+
11745	12397	A	emm59	ts	ST	+	+	-	+	-
11789	1568	A	MNT	ts	ST	+	+	-	+	-
11802	3266	A	MNT	ts	ST	+	+	-	+	-
11869	2950	A	ST4547	ts	ST	+	+	-	+	-
11961	4916	A	MNT	ts	ST	+	+	-	+	-
12015	12373	A	emm59	ts	ST	+	+	+	+	-
7625	8215	B	-	ts	ST	+	-	-	-	-
8011	3238	B	-	ts	ST	+	-	-	-	-
10388	1653	G	-	ts	ST	+	-	-	-	-
O12633	5395	B	-	ts	ST	+	-	-	-	-

Table 4: Genotyping of streptococcal isolates. The isolates were collected between 1976 and 1996 (group 1) and in 1998 (group 2) from patients with varying diseases. The results are based on PCR analysis using purified genomic DNA and specific primers for each of the sag genes.

The non Gas are: B, *S. agalactiae*; C, *S. equis*; G, *Streptococcus spp.*

MNT, M non typable: ts, throat site; ws, wound site; sk, skin; ps, pus site; hvs, high vaginal site; bc, blood culture; ST, sore throat; SF, scarlet fever; RF, rheumatic fever; AGN, acture glomerulonephritis; T carriage, throat carriage.

* and |, duplicate isolates; §, recently assigned as M89; II, recently assigned as M92.

INDUSTRIAL APPLICATION

5

The superantigens of the invention, polynucleotides which encode them and antibodies which bind them have numerous applications. A number of these are discussed above (including *Streptococci* subtyping, diagnostic applications and therapeutic applications) but it will be appreciated that these are but examples.

10 Other applications will present themselves to those skilled in the art and are in no way excluded from the scope of the invention.

It will also be appreciated that the foregoing examples are illustrations of the invention. The invention may be carried out with the numerous variations and
15 modifications as will be apparent to those skilled in the art. For example, a native superantigen may be replaced by a synthetic superantigen with on or more deletions, insertions and/or substitutions relative to the corresponding natural superantigen, provided that the superantigen activity is retained. Likewise there are many variations in the way in which the invention can be used in other aspects
20 of it.

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CLAIMS

1. A superantigen selected from any one of SMEZ-2, SPE-G, SPE-H and SPE-J, or a functionally equivalent variant thereof.
- 5 2. A superantigen which is SMEZ-2 and which has an amino acid sequence of SEQ ID NO. 2, or a functionally equivalent variant thereof.
3. A superantigen which is SPE-G and which has an amino acid sequence of
10 SEQ ID NO. 4, or a functionally equivalent variant thereof.
4. A superantigen which is SPE-H and which has an amino acid sequence of SEQ ID NO. 6, or a functionally equivalent variant thereof.
- 15 5. A superantigen which is SPE-J and which has an amino acid sequence which includes SEQ ID NO. 8, or a functionally equivalent variant thereof.
6. A polynucleotide comprising a nucleotide sequence encoding SMEZ-2 or a variant thereof as claimed in claim 2.
- 20 7. A polynucleotide according to claim 6 in which said nucleotide sequence is or includes SEQ ID NO. 1.
8. A polynucleotide comprising a nucleotide sequence encoding SPE-G or a
25 variant thereof as claimed in claim 3.
9. A polynucleotide according to claim 8 in which said nucleotide sequence is or includes SEQ ID NO. 3.
- 30 10. A polynucleotide comprising a nucleotide sequence encoding SPE-H or a variant thereof as claimed in claim 4.
11. A polynucleotide according to claim 10 in which said nucleotide sequence is or includes SEQ ID NO 5.

12. A polynucleotide comprising a nucleotide sequence encoding SPE-J or a variant thereof as claimed in claim 5.
13. A polynucleotide according to claim 12 in which said nucleotide sequence
5 includes SEQ ID NO. 7.
14. A method of subtyping *Streptococci* which includes the step of detecting the presence or absence of a superantigen as claimed in any one of claims 2 to 5.
- 10 15. A method of subtyping *Streptococci* which includes the step of detecting the presence or absence of a polynucleotide as claimed in any one of claims 6 to 13.
16. A construct which comprises a superantigen or variant thereof as claimed in any one of claims 2 to 5 and a cell-targeting molecule.
15
17. A construct according to claim 15 in which said cell-targeting molecule specifically binds a tumour cell.
18. A construct according to claim 15 or claim 16 in which said cell-targeting
20 molecule is an antibody.
19. A pharmaceutical composition which includes a construct as claimed in any one of claims 15 to 17.
- 25 20. An antibody which binds superantigen SMEZ-2 as claimed in claim 2.
21. An antibody which binds superantigen SPE-G as claimed in claim 3.
22. An antibody which binds superantigen SPE-H as claimed in claim 4.
30
23. An antibody which binds superantigen SPE-J as claimed in claim 5.
24. A kit which includes an antibody as claimed in any one of claims 19 to 22.
- 35 25. A nucleic acid molecule which hybridises to a polynucleotide of claim 7.

26. A nucleic acid molecule which hybridises to a polynucleotide of claim 9.
27. A nucleic acid molecule which hybridises to a polynucleotide of claim 11.
- 5 28. A nucleic acid molecule which hybridises to a polynucleotide of claim 13.
29. A kit which includes a nucleic acid molecule as claimed in any one of claims 25 to 28.
- 10 30. A method of diagnosing a disease which is caused or mediated by expression of a superantigen as claimed in claim 1 which includes the step of detecting the presence of said superantigen using an antibody as claimed in any one of claims 19 to 22, or detecting the presence of a polynucleotide encoding said superantigen using a nucleic acid molecule as claimed in any one of claims 25 to 15 28.

SEQUENCE LISTING

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	50					55				60						

aga	gat	gct	aga	gat	ttc	ttt	att	aac	tcc	gaa	atg	gac	gaa	tat	gca	240
Arg	Asp	Ala	Arg	Asp	Phe	Phe	Ile	Asn	Ser	Glu	Met	Asp	Glu	Tyr	Ala	
65					70					75				80		

gcc aat gat ttt aaa act gga gat aaa ata gct gtg ttc tcc gtc cca 288
 Ala Asn Asp Phe Lys Thr Gly Asp Lys Ile Ala Val Phe Ser Val Pro
 85 90 95

ttt gat tgg aac tat tta tca aaa gga aaa gtc aca gca tat acc tat 336
 Phe Asp Trp Asn Tyr Leu Ser Lys Gly Lys Val Thr Ala Tyr Thr Tyr
 100 105 110

ggg gga ata aca ccc tac caa aaa act tca ata cct aaa aat atc cct 384
 Gly Gly Ile Thr Pro Tyr Gln Lys Thr Ser Ile Pro Lys Asn Ile Pro
 115 120 125

gtt aat tta tgg att aat gga aag cag atc tct gtt cct tac aac gaa 432
 Val Asn Leu Trp Ile Asn Gly Lys Gln Ile Ser Val Pro Tyr Asn Glu
 130 135 140

ata tca act aac aaa aca aca gtt aca gct caa gaa att gat cta aag 480
 Ile Ser Thr Asn Lys Thr Thr Val Thr Ala Gln Glu Ile Asp Leu Lys
 145 150 155 160

gtt aga aaa ttt tta ata gca caa cat caa tta tat tct tct ggt tct 528
 Val Arg Lys Phe Leu Ile Ala Gln His Gln Leu Tyr Ser Ser Gly Ser
 165 170 175

agc tac aaa agt ggt aga ctg gtt ttt cat aca aat gat aat tca gat 576
 Ser Tyr Lys Ser Gly Arg Leu Val Phe His Thr Asn Asp Asn Ser Asp
 180 185 190

aaa tat tct ttc gat ctt ttc tat gta gga tat aga gat aaa gaa agt 624
 Lys Tyr Ser Phe Asp Leu Phe Tyr Val Gly Tyr Arg Asp Lys Glu Ser
 195 200 205

atc ttt aaa gta tac aaa gac aat aaa tct ttc aat ata gat aaa att 672
 Ile Phe Lys Val Tyr Lys Asp Asn Lys Ser Phe Asn Ile Asp Lys Ile
 210 215 220

ggg cat tta gat ata gaa att gac tcc taa 702
 Gly His Leu Asp Ile Glu Ile Asp Ser
 225 230

<210> 2

<211> 233

<212> PRT

<213> Streptococcus pyogenes

<400> 2

Met Lys Lys Thr Lys Leu Ile Phe Ser Phe Thr Ser Ile Phe Ile Ala

<210> 3
<211> 705

<212> DNA

<213> Streptococcus pyogenes

<220>

<221> CDS

<222> (1)..(702)

<400> 3

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atg aaa aca aac att ttg aca att atc ata tta tca tgt gtt ttt agc 48
Met Lys Thr Asn Ile Leu Thr Ile Ile Ile Leu Ser Cys Val Phe Ser
  1             5             10             15

tat gga agt caa tta gct tat gca gat gaa aat tta aaa gat tta aaa 96
Tyr Gly Ser Gln Leu Ala Tyr Ala Asp Glu Asn Leu Lys Asp Leu Lys
      20             25             30

aga agt tta aga ttt gcc tat aat att acc cca tgc gat tat gaa aat 144
Arg Ser Leu Arg Phe Ala Tyr Asn Ile Thr Pro Cys Asp Tyr Glu Asn
      35             40             45

gta gaa att gca ttt gtt act aca aat agc ata cat att aat act aaa 192
Val Glu Ile Ala Phe Val Thr Thr Asn Ser Ile His Ile Asn Thr Lys
      50             55             60

caa aaa aga tcg gaa tgt att ctt tat gtt gat tct att gta tct tta 240
Gln Lys Arg Ser Glu Cys Ile Leu Tyr Val Asp Ser Ile Val Ser Leu
      65             70             75             80

ggc att act gat cag ttt ata aaa ggg gat aag gtc gat gtt ttt ggt 288
Gly Ile Thr Asp Gln Phe Ile Lys Gly Asp Lys Val Asp Val Phe Gly
      85             90             95

ctc cct tat aat ttt tcc cca cct tat gta gat aat att tat ggt ggt 336
Leu Pro Tyr Asn Phe Ser Pro Pro Tyr Val Asp Asn Ile Tyr Gly Gly
      100             105             110

att gta aaa cat tcg aat caa gga aat aaa tca tta cag ttt gta gga 384
Ile Val Lys His Ser Asn Gln Gly Asn Lys Ser Leu Gln Phe Val Gly
      115             120             125

att tta aat caa gat ggg aaa gaa act tat ttg ccc tct gag gct gtt 432
Ile Leu Asn Gln Asp Gly Lys Glu Thr Tyr Leu Pro Ser Glu Ala Val
      130             135             140

cgc ata aaa aag aaa cag ttt act tta cag gaa ttt gat ttt aaa ata 480
Arg Ile Lys Lys Lys Gln Phe Thr Leu Gln Glu Phe Asp Phe Lys Ile
      145             150             155             160

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aga aaa ttt cta atg gaa aaa tac aat atc tat gat tcg gaa tcg cgt 528
 Arg Lys Phe Leu Met Glu Lys Tyr Asn Ile Tyr Asp Ser Glu Ser Arg
 165 170 175

tat aca tcg ggg agc ctt ttc ctt gct act aaa gat agt aaa cat tat 576
 Tyr Thr Ser Gly Ser Leu Phe Leu Ala Thr Lys Asp Ser Lys His Tyr
 180 185 190

gaa gtt gat tta ttt aat aag gat gat aag ctt tta agt cga gac agt 624
 Glu Val Asp Leu Phe Asn Lys Asp Asp Lys Leu Leu Ser Arg Asp Ser
 195 200 205

ttc ttt aaa agg tat aaa gat aat aag att ttt aat agt gaa gaa att 672
 Phe Phe Lys Arg Tyr Lys Asp Asn Lys Ile Phe Asn Ser Glu Glu Ile
 210 215 220

agt cat ttt gat atc tac tta aaa acg cac tag 705
 Ser His Phe Asp Ile Tyr Leu Lys Thr His
 225 230

<210> 4

<211> 234

<212> PRT

<213> Streptococcus pyogenes

<400> 4

Met Lys Thr Asn Ile Leu Thr Ile Ile Ile Leu Ser Cys Val Phe Ser
 1 5 10 15

Tyr Gly Ser Gln Leu Ala Tyr Ala Asp Glu Asn Leu Lys Asp Leu Lys
 20 25 30

Arg Ser Leu Arg Phe Ala Tyr Asn Ile Thr Pro Cys Asp Tyr Glu Asn
 35 40 45

Val Glu Ile Ala Phe Val Thr Thr Asn Ser Ile His Ile Asn Thr Lys
 50 55 60

Gln Lys Arg Ser Glu Cys Ile Leu Tyr Val Asp Ser Ile Val Ser Leu
 65 70 75 80

Gly Ile Thr Asp Gln Phe Ile Lys Gly Asp Lys Val Asp Val Phe Gly
 85 90 95

Leu Pro Tyr Asn Phe Ser Pro Pro Tyr Val Asp Asn Ile Tyr Gly Gly
 100 105 110

Ile Val Lys His Ser Asn Gln Gly Asn Lys Ser Leu Gln Phe Val Gly
 115 120 125

Ile Leu Asn Gln Asp Gly Lys Glu Thr Tyr Leu Pro Ser Glu Ala Val
 130 135 140

Arg Ile Lys Lys Lys Gln Phe Thr Leu Gln Glu Phe Asp Phe Lys Ile
 145 150 155 160

Arg Lys Phe Leu Met Glu Lys Tyr Asn Ile Tyr Asp Ser Glu Ser Arg
 165 170 175

Tyr Thr Ser Gly Ser Leu Phe Leu Ala Thr Lys Asp Ser Lys His Tyr
 180 185 190

Glu Val Asp Leu Phe Asn Lys Asp Asp Lys Leu Leu Ser Arg Asp Ser
 195 200 205

Phe Phe Lys Arg Tyr Lys Asp Asn Lys Ile Phe Asn Ser Glu Glu Ile
 210 215 220

Ser His Phe Asp Ile Tyr Leu Lys Thr His
 225 230

<210> 5

<211> 711

<212> DNA

<213> Streptococcus pyogenes

<220>

<221> CDS

<222> (1)..(708)

<400> 5

atg aga tat aat tgt cgc tac tca cat att gat aag aaa atc tac agc 48
 Met Arg Tyr Asn Cys Arg Tyr Ser His Ile Asp Lys Lys Ile Tyr Ser
 1 5 10 15

atg att ata tgt ttg tca ttt ctt tta tat tcc aat gtt gtt caa gca 96
 Met Ile Ile Cys Leu Ser Phe Leu Leu Tyr Ser Asn Val Val Gln Ala
 20 25 30

aat tct tat aat aca acc aat aga cat aat cta gaa tcg ctt tat aag 144
 Asn Ser Tyr Asn Thr Thr Asn Arg His Asn Leu Glu Ser Leu Tyr Lys
 35 40 45

cat gat tct aac ttg att gaa gcc gat agt ata aaa aat tct cca gat	192
His Asp Ser Asn Leu Ile Glu Ala Asp Ser Ile Lys Asn Ser Pro Asp	
50 55 60	
att gta aca agc cat atg ttg aaa tat agt gtc aag gat aaa aat ttg	240
Ile Val Thr Ser His Met Leu Lys Tyr Ser Val Lys Asp Lys Asn Leu	
65 70 75 80	
tca gtt ttt ttt gag aaa gat tgg ata tca cag gaa ttc aaa gat aaa	288
Ser Val Phe Phe Glu Lys Asp Trp Ile Ser Gln Glu Phe Lys Asp Lys	
85 90 95	
gaa gta gat att tat gct cta tct gca caa gag gtt tgt gaa tgt cca	336
Glu Val Asp Ile Tyr Ala Leu Ser Ala Gln Glu Val Cys Glu Cys Pro	
100 105 110	
ggg aaa agg tat gaa gcg ttt ggt gga att aca tta act aat tca gaa	384
Gly Lys Arg Tyr Glu Ala Phe Gly Gly Ile Thr Leu Thr Asn Ser Glu	
115 120 125	
aaa aaa gaa att aaa gtt cct gta aac gtg tgg gat aaa agt aaa caa	432
Lys Lys Glu Ile Lys Val Pro Val Asn Val Trp Asp Lys Ser Lys Gln	
130 135 140	
cag ccg cct atg ttt att aca gtc aat aaa ccg aaa gta acc gct cag	480
Gln Pro Pro Met Phe Ile Thr Val Asn Lys Pro Lys Val Thr Ala Gln	
145 150 155 160	
gaa gtg gat ata aaa gtt aga aag tta ttg att aag aaa tac gat atc	528
Glu Val Asp Ile Lys Val Arg Lys Leu Leu Ile Lys Lys Tyr Asp Ile	
165 170 175	
tat aat aac cgg gaa caa aaa tac tct aaa gga act gtt acc tta gat	576
Tyr Asn Asn Arg Glu Gln Lys Tyr Ser Lys Gly Thr Val Thr Leu Asp	
180 185 190	
tta aat tca ggt aaa gat att gtt ttt gat ttg tat tat ttt ggc aat	624
Leu Asn Ser Gly Lys Asp Ile Val Phe Asp Leu Tyr Tyr Phe Gly Asn	
195 200 205	
gga gac ttt aat agc atg cta aaa ata tat tcc aat aac gag aga ata	672
Gly Asp Phe Asn Ser Met Leu Lys Ile Tyr Ser Asn Asn Glu Arg Ile	
210 215 220	
gac tca act caa ttt cat gta gat gtg tca atc agc taa	711
Asp Ser Thr Gln Phe His Val Asp Val Ser Ile Ser	
225 230 235	

<210> 6

<211> 236

<212> PRT

<213> Streptococcus pyogenes

<400> 6

Met Arg Tyr Asn Cys Arg Tyr Ser His Ile Asp Lys Lys Ile Tyr Ser
1 5 10 15

Met Ile Ile Cys Leu Ser Phe Leu Leu Tyr Ser Asn Val Val Gln Ala
20 25 30

Asn Ser Tyr Asn Thr Thr Asn Arg His Asn Leu Glu Ser Leu Tyr Lys
35 40 45

His Asp Ser Asn Leu Ile Glu Ala Asp Ser Ile Lys Asn Ser Pro Asp
50 55 60

Ile Val Thr Ser His Met Leu Lys Tyr Ser Val Lys Asp Lys Asn Leu
65 70 75 80

Ser Val Phe Phe Glu Lys Asp Trp Ile Ser Gln Glu Phe Lys Asp Lys
85 90 95

Glu Val Asp Ile Tyr Ala Leu Ser Ala Gln Glu Val Cys Glu Cys Pro
100 105 110

Gly Lys Arg Tyr Glu Ala Phe Gly Gly Ile Thr Leu Thr Asn Ser Glu
115 120 125

Lys Lys Glu Ile Lys Val Pro Val Asn Val Trp Asp Lys Ser Lys Gln
130 135 140

Gln Pro Pro Met Phe Ile Thr Val Asn Lys Pro Lys Val Thr Ala Gln
145 150 155 160

Glu Val Asp Ile Lys Val Arg Lys Leu Leu Ile Lys Lys Tyr Asp Ile
165 170 175

Tyr Asn Asn Arg Glu Gln Lys Tyr Ser Lys Gly Thr Val Thr Leu Asp
180 185 190

Leu Asn Ser Gly Lys Asp Ile Val Phe Asp Leu Tyr Tyr Phe Gly Asn
195 200 205

Gly Asp Phe Asn Ser Met Leu Lys Ile Tyr Ser Asn Asn Glu Arg Ile
210 215 220

Asp Ser Thr Gln Phe His Val Asp Val Ser Ile Ser
 225 230 235

<210> 7

<211> 414

<212> DNA

<213> Streptococcus pyogenes

<220>

<221> CDS

<222> (1)..(411)

<400> 7

ctt ccg tac ata ttt act cgt tat gat gtt tat tat ata tat ggt ggg 48
 Leu Pro Tyr Ile Phe Thr Arg Tyr Asp Val Tyr Tyr Ile Tyr Gly Gly
 1 5 10 15

gtt aca cca tca gta aac agt aat tcg gaa aat agt aaa att gta ggt 96
 Val Thr Pro Ser Val Asn Ser Asn Ser Glu Asn Ser Lys Ile Val Gly
 20 25 30

aat tta cta ata gat gga gtc cag caa aaa aca cta ata aat ccc ata 144
 Asn Leu Leu Ile Asp Gly Val Gln Gln Lys Thr Leu Ile Asn Pro Ile
 35 40 45

aaa ata gat aaa cct att ttt acg att caa gaa ttt gac ttc aaa atc 192
 Lys Ile Asp Lys Pro Ile Phe Thr Ile Gln Glu Phe Asp Phe Lys Ile
 50 55 60

aga caa tat ctt atg caa aca tac aaa att tat gat cct aat tct cca 240
 Arg Gln Tyr Leu Met Gln Thr Tyr Lys Ile Tyr Asp Pro Asn Ser Pro
 65 70 75 80

tac ata aaa ggg caa tta gaa att gcg atc aat ggc aat aaa cat gaa 288
 Tyr Ile Lys Gly Gln Leu Glu Ile Ala Ile Asn Gly Asn Lys His Glu
 85 90 95

agt ttt aac tta tat gat gca acc tca tct agt aca agg agt gat att 336
 Ser Phe Asn Leu Tyr Asp Ala Thr Ser Ser Ser Thr Arg Ser Asp Ile
 100 105 110

ttt aaa aaa tat aaa gac aat aag act ata aat atg aaa gat ttc agc 384
 Phe Lys Lys Tyr Lys Asp Asn Lys Thr Ile Asn Met Lys Asp Phe Ser
 115 120 125

cat ttt gat att tac ctt tgg act aaa taa

414

His Phe Asp Ile Tyr Leu Trp Thr Lys

130

135

<210> 8

<211> 137

<212> PRT

<213> Streptococcus pyogenes

<400> 8

Leu Pro Tyr Ile Phe Thr Arg Tyr Asp Val Tyr Tyr Ile Tyr Gly Gly

1

5

10

15

Val Thr Pro Ser Val Asn Ser Asn Ser Glu Asn Ser Lys Ile Val Gly

20

25

30

Asn Leu Leu Ile Asp Gly Val Gln Gln Lys Thr Leu Ile Asn Pro Ile

35

40

45

Lys Ile Asp Lys Pro Ile Phe Thr Ile Gln Glu Phe Asp Phe Lys Ile

50

55

60

Arg Gln Tyr Leu Met Gln Thr Tyr Lys Ile Tyr Asp Pro Asn Ser Pro

65

70

75

80

Tyr Ile Lys Gly Gln Leu Glu Ile Ala Ile Asn Gly Asn Lys His Glu

85

90

95

Ser Phe Asn Leu Tyr Asp Ala Thr Ser Ser Ser Thr Arg Ser Asp Ile

100

105

110

Phe Lys Lys Tyr Lys Asp Asn Lys Thr Ile Asn Met Lys Asp Phe Ser

115

120

125

His Phe Asp Ile Tyr Leu Trp Thr Lys

130

135

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FIG 1

SMEZ	-----	-----	LEVDNNSL	NIYSTIVBY	SDTVIDE	30
SMEZ-2	-----	-----	LEVDNNSL	NIYSTIVBY	SDIVIDSK	30
SPE-J	-----	-----	-----	-----	-----	
SPE-C	-----	-----DSKK	DISNVKSD	YAYTITPYD	KDCRVNGS	34
SPE-G	-----	-----DE	NLKDLKRS	FAYNITPCD	ENVEIARV	32
SPE-H	-----	-----NSYN	TTNREHLE	YKHDSNLIE	DSIKNSPD	34
SEA	SEKSEEINE	KDLRKSELO	GAALGQLQ	YYNEKARTE	NKESHDQFL	49

 $\alpha 2$ $\beta 1$

SMEZ	BNLVTKRLDV	RDARDFFIN	EMDEYAAND	KDGRIAMES	VEFDWNLSE	80
SMEZ-2	BNLVTKRLDV	RDARDFFIN	EMDEYAAND	KTGRIAMES	VEFDWNLSE	80
SPE-J	-----	-----	-----	-----	-----	
SPE-C	HTLNIDTQKY	RG.KDYIIS	EMSYEASQ	KRDDHIVVEG	LF....MIFT	6
SPE-G	NSIHINTKQK	RSECILYVS	IVSLGITDQ	IKGKRVIVVEG	LF....MILN	79
SPE-H	TS.HML..KY	.SVKDKNLS	PFKDWISQ	FKDKEDIIYA	LSAQEVCE..	78
SEA	ETILFKGFFT	NHSWYNDLL	DFDSKDIDV	YKCHVLLYG	AYGYQCAGG	99

 $\beta 2$ $\beta 3$ $\alpha 3$ $\beta 4$

SMEZ	GKVIAH.TY	GMTPYQEE..	PMSKNIEV	WINRRQIPV	YQISTNKT	127
SMEZ-2	GKVIAH.TY	GMTPYQKT..	SILKNIEV	WINRRQISV	YQISTNKT	127
SPE-J	RYDVYV.IV	GVTPSVNSN	SENSRIVG	LDGQVQKTL	DPFKIDRPI	54
SPE-C	SHTGEY.IV	GLTPAQN.N	KVNEKLLG	FISGESQQNL	NKAILERDI	126
SPE-G	PPYVDN.IV	GLVKHSNQG	NKSLQFVG	NQDRETYLE	SEAVRIKPKQ	126
SPE-H	CPGKRTEAF	GLTLNSEK.	.KEIRVEVNV	WOKSKQQ..	PMF TVMRPK	124
SEA	TPNKTACMY	GVLEHDNRL	TEERKVEIN	GLD KQNTV	LETVKTERKN	149

 $\beta 5$ $\beta 6$ $\beta 7$ $\beta 8$

SMEZ	VTAQEDLKV	RPLISQHQ	SSGSSYKSG	KPVFHTDNS	DKYSUDIEYV	177
SMEZ-2	VTAQEDLKV	RPLIAQHQL	SSGSSYKSG	KPVFHTDNS	DKYSUDIEYV	177
SPE-J	FTIQEFDFRI	QYQMOTYKI	DPNSPYIKG	QPEIAINGNK	.HESFNLYDA	103
SPE-C	VTAQEDLKV	KVPMDNKYI	DATSPYVSG	RIEIGTRGK	.HEQIDHEDS	175
SPE-G	FALQEFDFRI	KQPMKYNKI	DSESRVYSG	STFLATKDSK	.HYEVHAKNK	175
SPE-H	VTAQEDLKV	RPLIKKYDI	NNR..EQKY	SKGTWLDLN	SGKDIVFDLY	172
SEA	VTVQELDLQA	RYLQEKYNL	NSDVFDGKV	QRGLIVFHTS	TEPSVNYDLF	199

 $\beta 8$ $\alpha 4$ $\beta 9$ $\beta 10$

SMEZ	..GYRDKESI	ERVYKRI	RSF	NIDKIGHLDI	EIDS	209
SMEZ-2	..GYRDKESI	ERVYKRI	RSF	NIDKIGHLDI	EIDS	209
SPE-J	TSS.STRSDI	ERKOKRI	RTI	DMKDFSHFDI	YIWTK	137
SPE-C	PNE.GTRSDI	SAKYRI	RRI	MMKNFSHFDI	YTERK	208
SPE-G	DDKLLSRDSF	ERVYKRI	KIF	MSSEEISHFDI	YKTH	210
SPE-H	YFGNGDFNSM	LKIDYSN	ERI	DSTQF.HVDV	SIS	204
SEA	CAQGQNSNTL	LRIYRI	RTI	MSSENH.HID	YIYTS	233

 $\alpha 5$ $\beta 11$ $\beta 12$

FIG 2

10 30 50
ATGAAAAAACAAACTTATTTTTCTTTTACTTCAATATTCATTGCAATAATTTCTCGT
M K K T K L I F S F T S I F I A I I S R

70 90 110
CCTGTGTTTGGATTAGAAGTAGATAATAATTCCCTTCTAAGGAATATCTATAGTACGATT
P V F G L E V D N N S L L R N I Y S T I

130 150 170
GTATATGAATATTCAGATATAGTAATTGATTTTAAAACCAGTCATAACTTAGTGACTAAG
V Y E Y S D I V I D F K T S H N L V T K

190 210 230
AAACTTGATGTTAGAGATGCTAGAGATTTCTTTATTAACCTCCGAAATGGACGAATATGCA
K L D V R D A R D F F I N S E M D E Y A

250 270 290
GCCAATGATTTTAAAACCTGGAGATAAAATAGCTGTGTTCTCCGTCCCATTGATTGGAAC
A N D F K T G D K I A V F S V P F D W N

310 330 350
TATTTATCAAAAGGAAAAGTCACAGCATATACCTATGGTGAATAACACCCTACCAAAAA
Y L S K G K V T A Y T Y G G I T P Y Q K

370 390 410
ACTTCAATACCTAAAAAatCCCTGTTAATTTATGGattaatGgAAAGcagatCTCTgtT
T S I P K N I P V N L W I N G K Q I S V

430 450 470
CcTtaCaaCGAAATATCaaCTAACAAAACAacaGTTACAGCTCAAGAAAttgatCTAAAG
P Y N E I S T N K T T V T A Q E I D L K

490 510 530
GTTAGAAAATTTTAAATAGCACAAACATCAATTATATTCTTCTGTTCTAGCTACAAAAGT
V R K F L I A Q H Q L Y S S G S S Y K S

550 570 590
GGTAGACTGGTTTTTCATACAAATGATAATTCAGATAAATATTCTTTTCgattcTTTctat
G R L V F H T N D N S D K Y S F D L F Y

610 630 650
gtagGATATAGAGATAAAGAAAGTATCTTTAAAGTATACAAAGACAATAAATCTTTCAAT
V G Y R D K E S I F K V Y K D N K S F N

670 690
ATAGATAAAATGGGCATTAGATATAGAAATTGACTCCTAA
I D K I G H L D I E I D S *

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SPE-G

FIG 3

10 30 50
ATGAAACAAACATTTTGACAATTATCATATTATCATGTGTTTTAGCTATGGAAGTCAA
M K T N I L T I I I L S C V F S Y G S Q

70 90 110
TTAGCTTATGCAGATGAAAATTTAAAAGATTTAAAAAGAAGTTTAAGATTGCCTATAAT
L A Y A D E N L K D L K R S L R F A Y N

130 150 170
ATTACCCCATGCGATTATGAAAATGTAGAAATTGCATTTGTTACTACAAATAGCATAACAT
I T P C D Y E N V E I A F V T T N S I H

190 210 230
ATTAATACTAAACAAAAAGATCGGAATGTATTCTTTATGTTGATTCTATTGTATCTTTA
I N T K Q K R S E C I L Y V D S I V S L

250 270 290
GGCATTACTGATCAGTTTATAAAAGGGGATAAGGTCGATGTTTTGGTCTCCCTTATAAT
G I T D Q F I K G D K V D V F G L P Y N

310 330 350
TTTTCCCCACCTTATGTAGATAATATTTATGGTGGTATTGTAAACATTCTGAATCAAGGA
F S P P Y V D N I Y G G I V K H S N Q G

370 390 410
AATAAATCATTACAGTTTGTAGGAATTTTAAATCAAGATGGGAAAGAACTTATTTGCCC
N K S L Q F V G I L N Q D G K E T Y L P

430 450 470
TctgAGGCTGTTCGCATAAAAAAGAAACAGTTTACTTTACAGGAATtgATTTTAAATA
S E A V R I K K K Q F T L Q E F D F K I

490 510 530
AGAAAATTTCTAATGGAAAAATACAATATCTATGATTGCGGAATCGCGTTATACATCGGGG
R K F L M E K Y N I Y D S E S R Y T S G

550 570 590
AGCCTTTTCCTTGCTACTAAAGATAGTAAACATTATGAAGTTGATTATTTAATAAGGAT
S L F L A T K D S K H Y E V D L F N K D

610 630 650
GATAAGCTTTTAAGTCGAGACAGTTTCTTTAAAAGGTATAAAGATAATAAGATTTTAAAT
D K L L S R D S F F K R Y K D N K I F N

670 690
AGTGAAGAAATTAGTCATTTTGATATCTACTTAAAAACGCACTAG
S E E I S H F D I Y L K T H *

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SPE-H

FIG 4

10 30 50
ATGAGATATAATTGTCGCTACTCACATATTGATAAGAAAATCTACAGCATGATTATATGT
M R Y N C R Y S H I D K K I Y S M I I C

70 90 110
TTGTCATTTCCTTTATATTCCAATGTTGTTCAAGCAAATTCTTATAATACAACCAATAGA
L S F L L Y S N V V Q A N S Y N T T N R

130 150 170
CATAATCTAGAATCGCTTTATAAGCATGATTCTAACTTGATTGAAGCCGATAGTATAAAA
H N L E S L Y K H D S N L I E A D S I K

190 210 230
AATTCTCCAGATATTGTAACAAGCCATATGTTGAAATATAGTGTCAAGGATAAAAATTTG
N S P D I V T S H M L K Y S V K D K N L

250 270 290
TCAGTTTTTTTTGAGAAAGATTGGATATCACAGGAATTCAAAGATAAAGAAGTAGATATT
S V F F E K D W I S Q E F K D K E V D I

310 330 350
TATGCTCTATCTGCACAAGAGGTTTGTGAATGTCCAGGAAAAGGTATGAAGCGTTtggt
Y A L S A Q E V C E C P G K R Y E A F G

370 390 410
GGAATTACATTAACCTAATTCAGAAAAAAGAAATTAAAGTTCCTGTAAACGtgtGggat
G I T L T N S E K K E I K V P V N V W D

430 450 470
AAAAGTAAACAACAGCCGCCTATGTTTATTACAGTCAATAAACCGAAagtaaCCGCTCAG
K S K Q Q P P M F I T V N K P K V T A Q

490 510 530
GAAGTGGATATAAAAGTTAGAAAGTTATTGAttaagaaatacgATATCTATAATAaccgg
E V D I K V R K L L I K K Y D I Y N N R

550 570 590
gaacaaaaataactctaaagggaactgttaccttagATTTAAATTCAGGTAAAGATATTGTT
E Q K Y S K G T V T L D L N S G K D I V

610 630 650
TTTGATTGTATTATTTTGGCAATGGAGACTTTAATAGCATGCTAAAAATATATTCCAAT
F D L Y Y F G N G D F N S M L K I Y S N

670 690 710
AACGAGAGAATAGactcaactCAATTTTCATGTAGatgTGTaatcagctaA
N E R I D S T Q F H V D V S I S *

SPE-J (partial)

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FIG 5

10 30 50
CTTCCGTACATATTTACTCGTTATGATGTTTATTATATATATGGTGGGGTTACACCATCA
L P Y I F T R Y D V Y Y I Y G G V T P S

70 90 110
GTAACAGTAATTCGGAAAATAGTAAAATTGTAGGTAATTTACTAATAGATGGAGTCCAG
V N S N S E N S K I V G N L L I D G V Q

130 150 170
CAAAAAACACTAATAAATCCCATAAAAATAGATAAACCTATTTTACGATTCAAGAATTT
Q K T L I N P I K I D K P I F T I Q E F

190 210 230
GACTTCAAAATCAGACAATATCTTATGCAAACATACAAAATTTATGATCCTAATTCTCCA
D F K I R Q Y L M Q T Y K I Y D P N S P

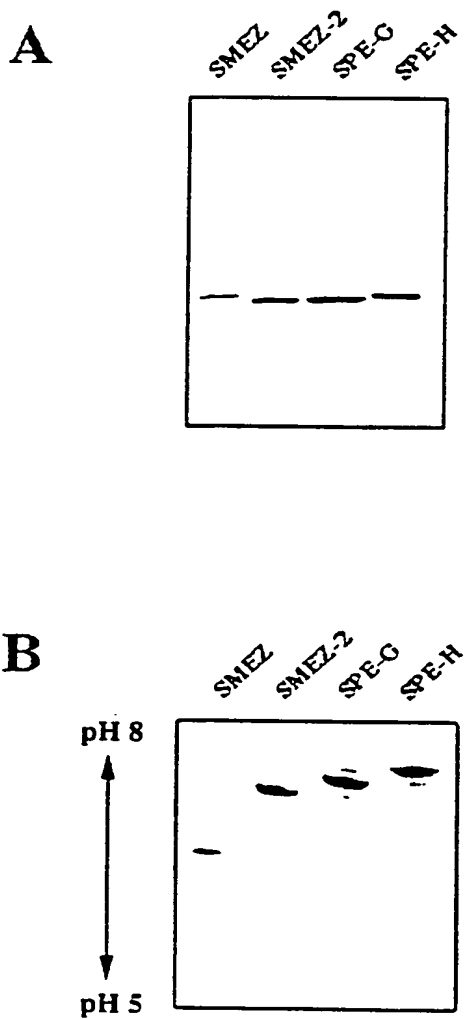
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Y I K G Q L E I A I N G N K H E S F N L

310 330 350
TATGATGCAACCTCATCTAGTACAAGGAGTGATATTTTAAAAAATATAAGACaATAAG
Y D A T S S S T R S D I F K K Y K D N K

370 390 410
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T I N M K D F S H F D I Y L W T K *

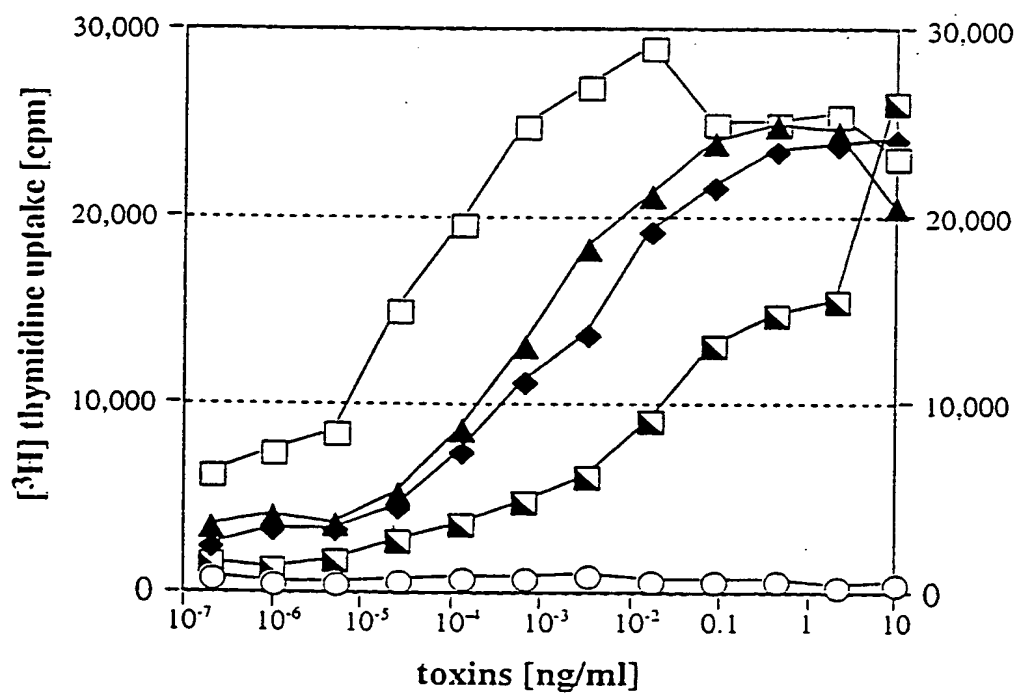
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FIG 6



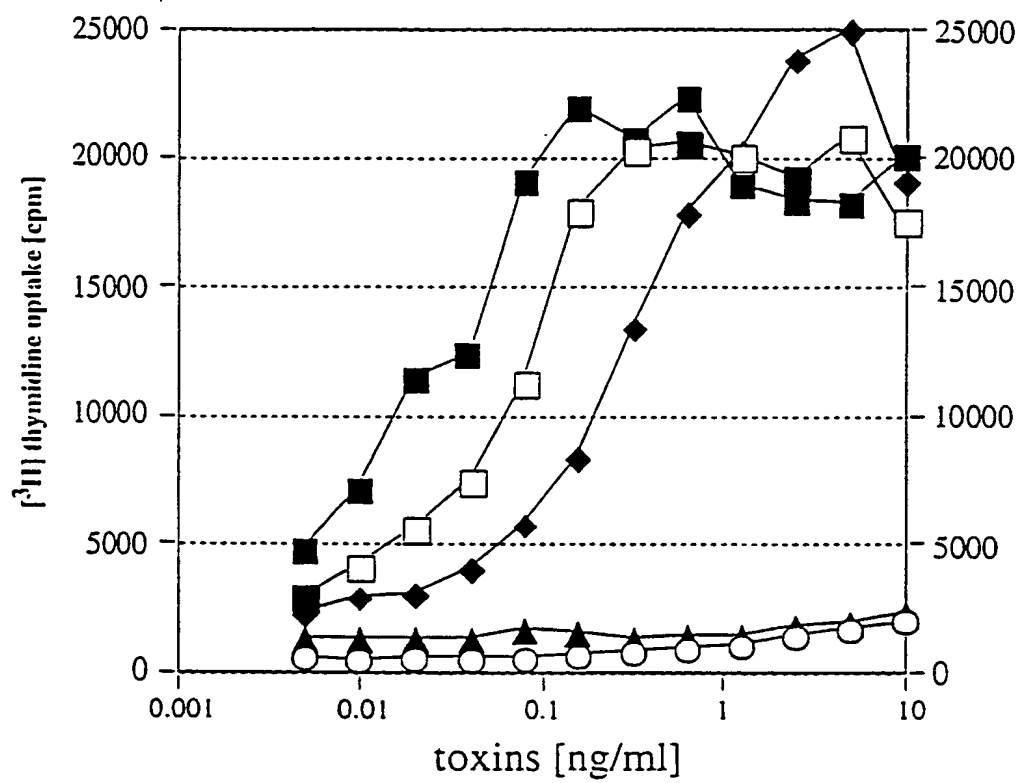
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FIG 7



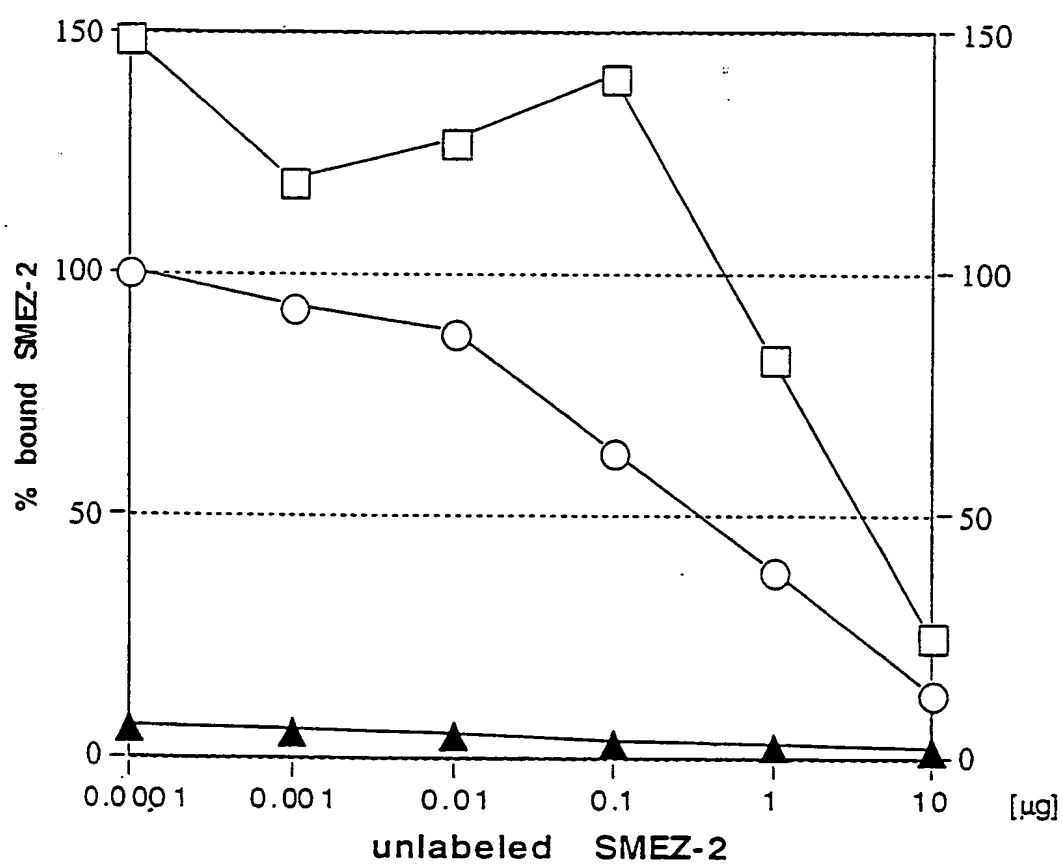
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FIG 8



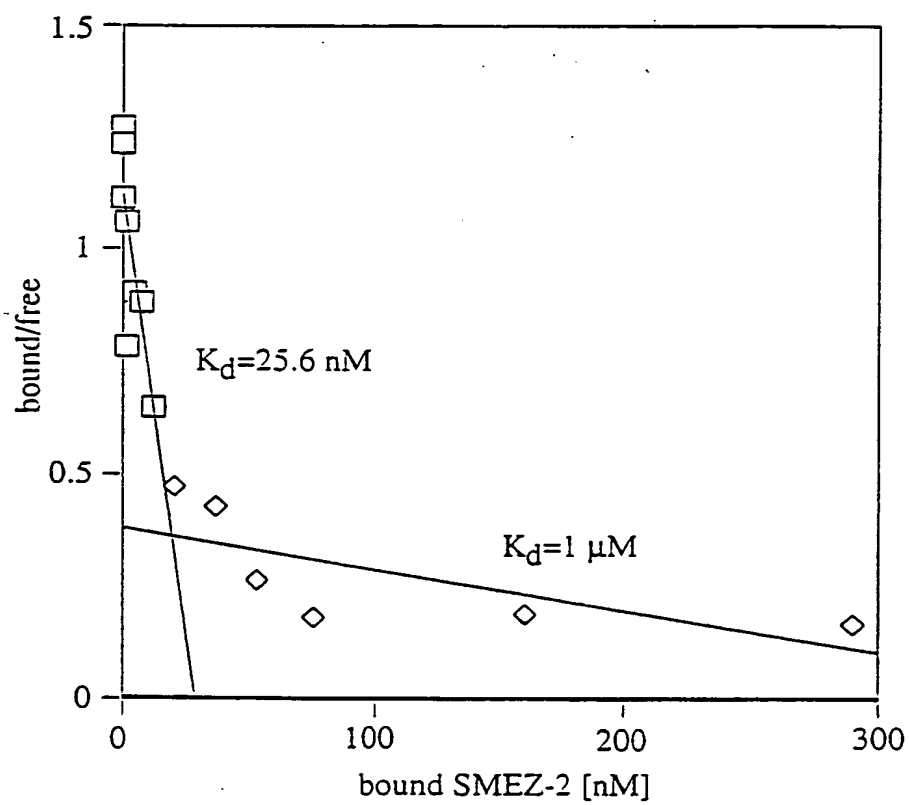
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FIG 9



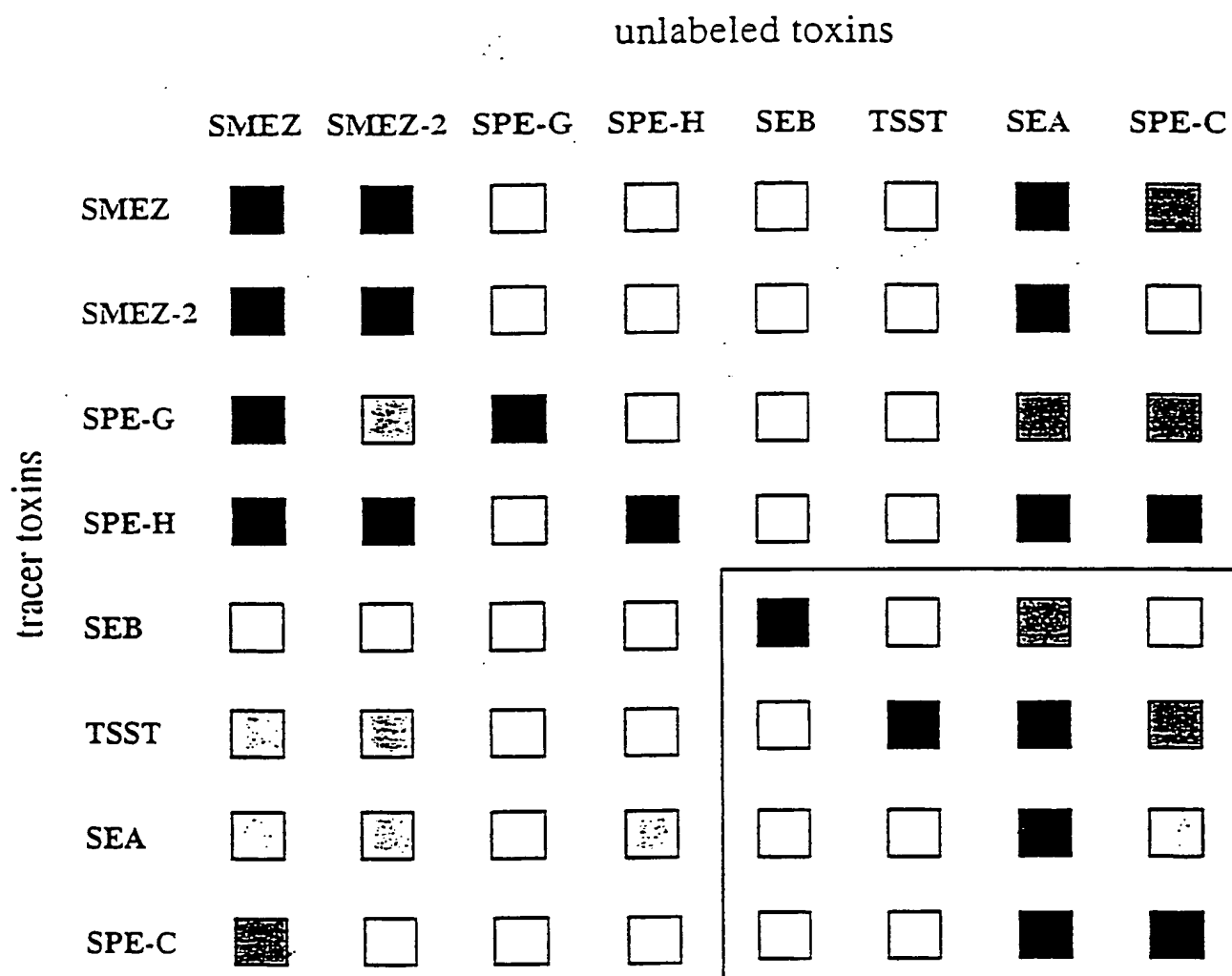
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FIG 10



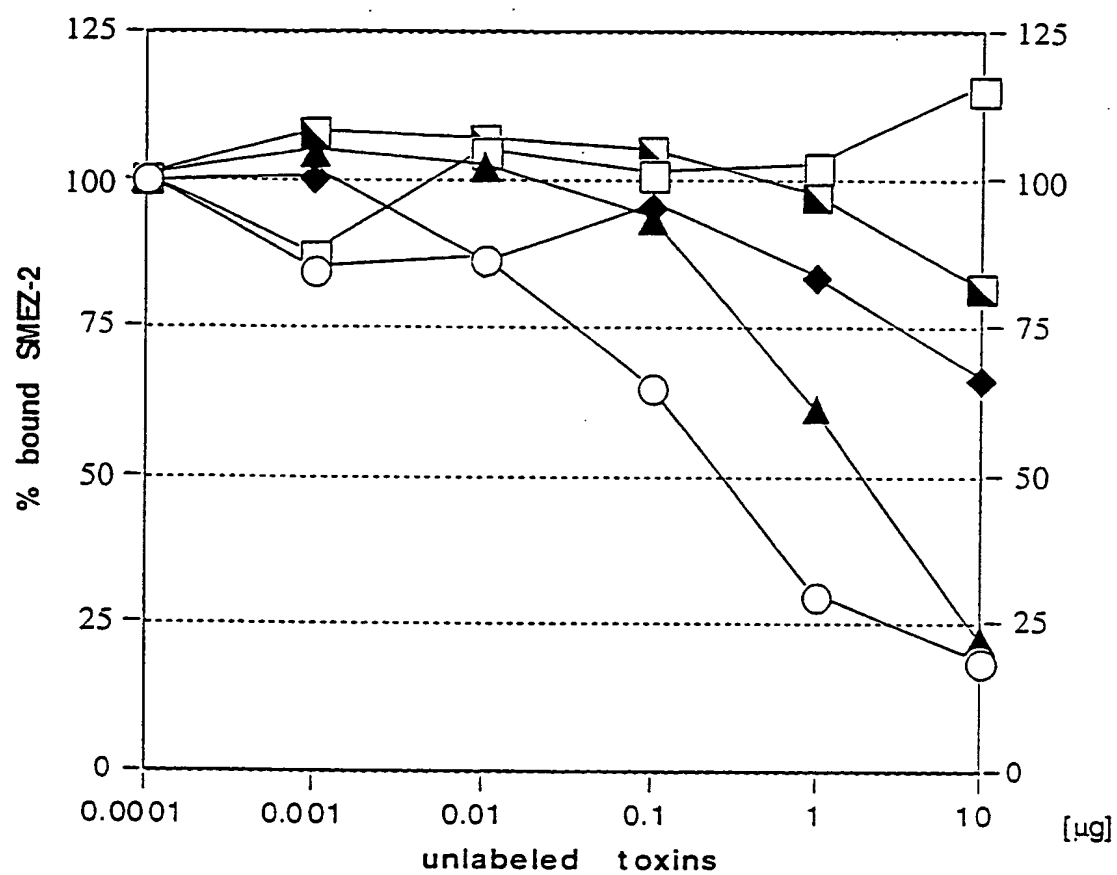
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FIG 11



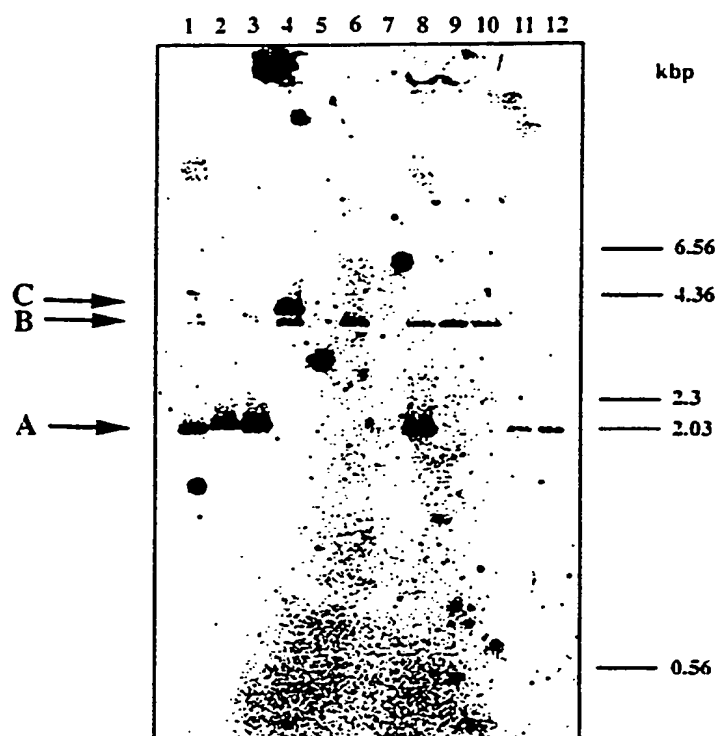
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FIG 12



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FIG 13



INTERNATIONAL SEARCH REPORT

International application No.
PCT/NZ99/00228**A. CLASSIFICATION OF SUBJECT MATTER**Int. Cl. ⁷: C07K 14/315, C07K 16/12, C07H 19/00, C12N 1/20, C12Q 1/68, A61K 35/74, A61K 39/09

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

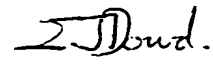
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
STN: File WPIDS Keywords used: "superantigen or super(w) antigen" and "streptococ?"
ANGIS Database: Sequence ID No's 2, 4, 6, and 8.**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	Journal of Experimental medicine (1999) 189(1), 89-101 "Identification and characterization of novel superantigens from <i>Streptococcus pyogenes</i> " Proft, T et al	1-30
P,X	Database GenPept, Accession No. CAB51744, Authors: Gerlach, D, Wagner, M, Fleischer, B, Schmidt, KH, Vettermann, S, Reichardt, W. Submitted 29 July 1999.	1, 2, 6, 7, 14-20, 24, 25, 29, 30
P,X	Database GenPept, Accession No. CAB51332, Authors: Gerlach, D, Wagner, M, Fleischer, B, Schmidt, KH, Vettermann, S, Reichardt, W. Submitted 19 July 1999.	1, 2, 6, 7, 14-20, 24, 25, 29, 30

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	Database GenPept, Accession No. CAB51142, Authors: Gerlach, D, Wagner, M, Fleischer, B, Schmidt, KH, Vettermann, S, Reichardt, W. Submitted 19 July 1999.	1, 2, 6, 7, 14-20, 24, 25, 29, 30
X	Infection and Immunity (1998) 66(7), 3337-3348 " Identification and Characterization of Staphylococcal Enterotoxin Types G and I from <i>Staphylococcus aureus</i> " Munson, SH et al.	1, 2, 6, 7, 14-20, 24, 25, 29, 30
X	Molecular Microbiology (1998) 29(2), 527-543 "The gene for toxic shock toxin is carried by a family of mobile pathogenicity islands in <i>staphylococcus aureus</i> ". Lindsay, JA et al.	1, 2, 6, 7, 14-20, 24, 25, 29, 30
X	Infection and Immunity (1998) 56(9), 2518-2520 " Nucleotide Sequence of Streptococcal Pyrogenic Exotoxin Type C" Goshorn, SC and Schlievert, PM.	1, 5, 12-20, 23, 24, 28-30
X	Database Swiss-Prot, Accession No. SPEC_STRPY & Infection and Immunity (1998) 56(9), 2518-2520 & Infection and Immunity (1992) 60: 3513-3517 & Nat Struct Biol (1997) 4: 635-643	1, 5, 12-20, 23, 24, 28-30
X	Database GenPept, Accession No. AAB 59091, & Infection and Immunity (1998) 56(9), 2518-2520 & Infection and Immunity (1992) 60: 3513-3517	1, 5, 12-20, 23, 24, 28-30
P,X	WO 99/27889 (10June 1999) IDAHO RESEARCH FOUNDATION INC See claim 3 in particular.	1, 3, 4, 8-11, 14-19, 21-22, 24, 26-27, 29-30

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/NZ99/00228

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Patent Document Cited in Search Report	Patent Family Member
WO 99/27889	
END OF ANNEX	



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : C07K 14/315, 16/12, C07H 19/00, C12N 1/20, C12Q 1/68, A61K 35/74, 39/09	A1	(11) International Publication Number: WO 00/39159 (43) International Publication Date: 6 July 2000 (06.07.00)
(21) International Application Number: PCT/NZ99/00228 (22) International Filing Date: 24 December 1999 (24.12.99) (30) Priority Data: 333589 24 December 1998 (24.12.98) NZ (71) Applicant (for all designated States except US): AUCKLAND UNISERVICES LIMITED [NZ/NZ]; 58 Symonds Street, Auckland (NZ). (72) Inventors; and (75) Inventors/Applicants (for US only): FRASER, John, David [NZ/NZ]; Auckland UniServices Limited, UniServices House, 58 Symonds Street, Auckland (NZ). PROFT, Thomas [NZ/NZ]; Auckland UniServices Limited, UniServices House, 58 Symonds Street, Auckland (NZ). (74) Agents: BENNETT, Michael, Roy et al.; West-Walker Bennett, Mobil on the Park, 157 Lambton Quay, Wellington (NZ).		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: SUPERANTIGENS (57) Abstract <p>The invention provides superantigens SMEZ-2, SPE-G, SPE-H and SPE-J, as well as polynucleotides which encode them. Such superantigens have, <i>inter alia</i>, diagnostic and therapeutic applications.</p>		

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